“A COMPARATIVE EVALUATION OF ANTIMICROBIAL EFFICACY OF FOSFOMYCIN AND CALCIUM HYDROXIDE WITH CHLORHEXIDINE AGAINST ENTEROCOCCUS FAECALIS IN RETREATMENT CASES USING COLONY FORMING UNITS- A RANDOMIZED CONTROL TRIAL”

DISSERTATION

Submitted to

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In Partial Fulfilment of the Requirement for the award of the degree of

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CONSERVATIVE DENTISTRY AND ENDODONTICS

SANTOSH
DENTAL COLLEGE & HOSPITAL

Pratap Vihar Sector- 12 Ghaziabad 201009
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BY

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INTRODUCTION

Successful endodontic treatment is subjected on the reduction in the bacterial load of the root canal and to minimize the reoccurrence of bacteria into the canal. The complex anatomical variation in the tooth and the ability of certain bacteria to persist in unfavorable conditions make complete disinfection challenging. Among the microbial species associated with endodontic failures, *Enterococcus faecalis* has garnered significant attention due to its increased potential to resist the commonly placed medicaments inside the canal and its ability to inhabit in a nutrient-deprived environments. This has led to a continuous search for more effective antimicrobial agents that can eliminate *E. faecalis* during endodontic retreatment procedures.

The root canal treatment involves multiple steps, including removing pulp, biomechanical preparation, sealing the radicular canal space followed by a permanent filing for the tooth [3]. The goal of root canal treatment is to establish a clean and bacteria-free space conducive to proper healing by entirely eliminating the bacteria from the canal.

The main purpose of treating the root canal is to systematically provide a thoroughly cleaned and properly shaped root canal and to seal it to prevent reinfection [1]. Treatment failure occurs when it does not meet the required standards [1]. Retreating the root canal treated tooth can be due to developing a condition requiring further endodontic intervention for a favorable prognosis. The primary reason for an unsuccessful endodontic therapy is inadequate biomechanical preparation and improper obturation [2]. The failure of endodontic therapy can also be caused by Intraradicular – Persistent Microorganisms and Procedural Errors and Extra radicular infections – Non microbial causes [3].

Contrasting to the teeth undergoing first time RCT (polymicrobial and primarily consist of gram-positive anaerobic rods) secondary infections typically involve few bacterial species. *Enterococcus faecalis* is a resilient bacterium, although present in small numbers in

untouched canals, has a major part in the development of obstinate peri-radicular lesions after the treatment. It is frequently seen in cases of RCT failure and can last within the canal either as a solitary organism or as a dominant member of the microbial community [4]. According to multiple investigations, E. faecalis was more frequently detected in endodontic treatment failure patients than in initial infection cases. With prevalence rates reaching as high as 90%, E. faecalis has been recognized as the leading cause of pain and infection following endodontic therapy in all documented cases. Among all patients with primary endodontic infections, E. faecalis was more commonly associated with asymptomatic cases than with symptomatic ones [4].

E. faecalis is found in numerous retreatment cases [5]. This observation can be understood through several survival and virulence characteristics of E. faecalis, including its ability to surpass competing microorganisms, penetrate dentinal tubules, and endure a lack of nutrients.[5]. This gram-positive bacterium with a size of 0.6µm-2.5µm [6,7] tends to enter dentinal tubules (1µm - 8µm) till a depth of almost 1200µm [6]. The present day intracanal medicaments like Calcium Hydroxide, Triple Antibiotic Paste have failed to eliminate these bacteria completely from the dentinal tubules [6]. These bacteria as mentioned earlier are resistant to various intracanal medicaments [6].

A medicament is a potent antimicrobial agent applied inside the canal between treatment sessions to eliminate any remaining microorganisms and inhibit the growth of new ones[4].In recent years, attention has turned toward exploring novel antimicrobial agents with improved efficacy against resistant endodontic pathogens. Fosfomycin, a broad-spectrum bactericidal antibiotic, has shown promising results due to its unique mechanism of working, targeting the MurA enzyme in the early stages, responsible for bacterial cell wall synthesis[8]. Its low molecular weight and hydrophilic nature may allow it to penetrate into dentinal tubules more effectively, potentially overcoming the limitations faced by traditional medicaments. Furthermore, fosfomycin has demonstrated potent activity against gram-positive bacteria, including *E. faecalis*, making it a candidate worth evaluating in endodontic applications[9]

Fosfomycin, a broad-spectrum bactericidal antibiotic., was discovered in 1969 at the Spanish Penicillin and Antibiotics Company and is made by the bacterium Streptomyces fradiae [8]. It is a FDA-approved drug with a trade name of "MONUROL", effective against both gram + and gram - bacteria [9]. It is widely employed in treating E. coli and E. faecalis-related uncomplicated urinary tract infections

Fosfomycin can also be produced synthetically [8]. For oral administration, it comes as calcium or trometamol salt [8] and for intravenous administration [8] it comes as a disodium salt. An oral dosage of 3 grammes is advised based on the safety profile [9]. Chemically, it prevents the production of the cell wall in its intital stages by inhibiting the precursor UDP-MurNAc formation which is considered as the primary stage in the formation of microbial cell wall [1]0. It catalyzes the transmission of the Enol pyruvyl fraction of PEP to the 3′-hydroxyl group of UDP-N-acetylglucosamine, UDP-N-acetylglucosamine enol pyruvyl transferase (MurA) is specifically engaged in peptidoglycan biosynthesis. Fosfomycin inactivates MurA by attaching to the thiol group of a cysteine [11]. Compared to the actions of other drugs, this inhibitory action of this drug occurs early [11]. Fosfomycin, in addition, decreases bacterial adhesion to epithelial cells and also inhibits platelet activator factor, which lessens its adherence of other bacteria like Haemophilus influenzae and Streptococcus pneumoniae [12].

The factors such as higher efficacy of Fosfomycin against resistant microbes like E.Faecalis in Urinary Track Infections has lead us to design our study to evaluate its effectiveness in retreatment cases.Despite the promising in vitro results, there is a paucity of clinical trials evaluating the efficacy of fosfomycin in endodontic retreatment scenarios. The existing literature is restricted and frequently misses direct comparisons with well-established medications like calcium hydroxide and chlorhexidine. Due to the high recurrence rate of E. faecalis in unsuccessful root canal treatments and the increasing resistance to traditional agents, it is crucial to investigate newer options and evaluate their effectiveness in a controlled clinical environment. The use of colony-forming unit (CFU) analysis remains one of the most reliable quantitative methods to assess microbial load and monitor the

effectiveness of intracanal medicaments. By evaluating CFU counts pre- and post-intervention, clinicians can obtain objective insights into the antimicrobial performance of various agents in vivo.

Consequently, the current randomized controlled trial seeks to assess the antibacterial effectiveness of fosfomycin and calcium hydroxide in combination with chlorhexidine against Enterococcus faecalis in the retreatment of single-rooted teeth, employing CFU analysis. By the end of this study we are expecting to provide valuable evidence regarding the potential use of fosfomycin as an intracanal medicament and its effectiveness in overcoming microbial resistance, thereby contributing to improved clinical outcomes in endodontic retreatment.

AIMS AND OBJECTIVES

AIM OF THE STUDY-

To evaluate and compare the Antimicrobial Efficacy of Fosfomycin and Calcium Hydroxide with Chlorhexidine gel (2%) against E faecalis in teeth requiring endodontic retreatment by evaluating the reduction in Colony Forming Units.

OBJECTIVE OF THE STUDY

- To evaluate the antimicrobial efficacy of Fosfomycin as an intracanal medicament in retreatment cases of single rooted teeth against E.faecalis using Colony Forming Units.
- To evaluate the antimicrobial efficacy of Calcium Hydroxide powder + Chlorhexidine gel (2%) as an intracanal medicament in retreatment cases of single rooted teeth using Colony Forming Units.
- To evaluate and compare the antimicrobial efficacy of Fosfomycin and $Ca(OH)_2$ + Chlorhexidine (2%) as an intracanal medicament in retreatment cases using Colony Forming Units.

REVIEW OF LITERATURE

1. ***Heiling et al and coworkers (1992)***[10] This study evaluated the antibacterial efficacy of an alkaline calcium based medicament and a chlorhexidine-based prolonged release device in eliminating as well as preventing bacterial infection. *Streptococcus faecalis* inoculated bovine dentin samples were used. The experiment compared two variations of the SRD, which differed in their cross-linking properties, along with $Ca(OH)_2$ and normal saline as a control. The ability of each material to eliminate existing bacteria and prevent reinfection was specifically assessed at 72 hours and 7 days. The findings showed that both SRD formulations were highly effective in significantly lowering bacterial counts in samples with primary infections and successfully prevented reinfection in previously sterilized dentin. In contrast, calcium hydroxide did not demonstrate notable antimicrobial action and was unable to clean the dentinal tubules or protect them from secondary contamination during the time points evaluated.

2. ***M.Evans et al and coworkers (2002)***[11] This research examined the survival strategies of Enterococcus faecalis under high levels of calcium hydroxide, concentrating on the importance of stress-related protein production and the function of the proton pump. The bacteria showed resistance at around pH = 11. Pre-exposure to a lower pH did not increase tolerance to higher pH levels. Blocking protein synthesis had no effect on bacterial survival, whereas inhibition of the proton pump significantly reduced viability. The findings suggest that *E. faecalis* survival in high-pH environments is dependent on an active proton pump rather than stress-induced protein responses.

3. ***ET Pinhero et al and coworkers (2003)***[12] examined the microbial communities in root canals of teeth with unsuccessful endodontic treatments reveal a distinct composition compared to those in untreated necrotic pulps. This specific study included 30 teeth that had undergone root canal treatment and still exhibited persistent periapical lesions to identify the bacterial composition and evaluate antibiotic resistance. Specialized techniques for anaerobic bacteria were employed in this analysis, 55 bacterial species were identified—most of them (80%) were gram + , and 58% were facultative anaerobes. The dominant genera included *Enterococcus*, *Streptococcus*, *Pepto streptococcus* and *Actinomyces*. Antibiotic susceptibility testing, conducted via the E-test, showed that *E. faecalis* and *Peptostreptococcus* were sensitive to penicillin-based antibiotics, while *E. faecalis* displayed resistance in 20% of isolates to erythromycin and in 60% to azithromycin. These results highlight a predominance of gram-positive, facultative anaerobes—particularly *E. faecalis*—in persistent endodontic infections, with some resistance to macrolides.

4. ***Joseph A .et al and co-workers (2005)***[13] evaluated the disinfecting potential of calcium and hydroxide mixed with 2% chlorhexidine (CHX) compared to $Ca(OH)_2$ mixed with water in retreatment cases of failed root canal therapy. Forty single-rooted teeth with existing periradicular lesions that had previously undergone root canal treatment were included. Each tooth was nonsurgically retreated and received intracanal medication during three appointments spaced 7–10 days apart. The two groups were medicated with either a $Ca(OH)_2$ and water slurry or a combination of $Ca(OH)_2$ and 2% chlorhexidine. Microbial cultures from the root canals were collected in thioglycolate and monitored for turbidity daily for the first week and weekly for the following three weeks. Initial testing showed that 30% of the samples (12 out of 40 teeth) had bacterial presence prior to retreatment. In the group treated with $Ca(OH)_2$ and water, 12 out of 20 teeth (60%) showed complete disinfection, including 2 of 4 teeth initially testing positive for *Enterococcus* species.

In contrast, the group treated with $Ca(OH)_2$ and CHX achieved disinfection in 16 out of 20 cases (80%) by the third visit. These findings suggest that combining 2% chlorhexidine with calcium hydroxide may enhance antimicrobial effectiveness during endodontic retreatment

5. ***Stuart et al and coworkers (2005)***[14] had concluded that *Enterococcus faecalis* is frequently found in persistent, symptom-free endodontic infections, its occurrence reported in 24% to 77% of such cases. This bacterium was notably resilient due to its unique virulence and survival mechanisms, including its ability to outcompete other microbes, penetrate dentinal tubules, and endure periods of limited nutrients. Current strategies to effectively manage *E. faecalis* in root canal systems emphasize strict aseptic protocols, enhanced apical enlargement during instrumentation, and the combined use of 2% chlorhexidine with sodium hypochlorite. As dental practices evolve, ongoing research focused on eliminating *E. faecalis* is likely to play a vital role in shaping the future of endodontic treatment.

6. **Kaufmann and coworkers (2006)**[15] conducted a research to find the presence of *Enterococcus* species in previously root canal treated teeth and compared cases with periradicular lesions to those without such lesions that still required retreatment. A total of 58 teeth, all of which had undergone endodontic therapy more than a year prior and now required retreatment, were examined. DNA extraction followed by PCR was used to identify bacterial presence. The findings revealed that bacteria were present in 90% of the samples, but *Enterococcus* species were detected in only 12% of cases. These results suggest that while bacterial contamination is strongly linked to endodontic failure, *Enterococcus* species alone may not be directly associated with the presence of periradicular pathology.

7. ***B Athanassiadis and coworkers (2007)***[16] had suggested in their study that bacteria are a normal finding during the development of pulp and periodontal diseases, and the goal of disinfecting the root canal is to eradicate these bacteria and create uninhabitable surroundings for the bacteria. This can be managed through aseptic techniques, chemomechanical preparation, antimicrobial irrigation, and intracanal medicaments. The choice of medicament depends on the diagnosis and understanding of the bacteria involved. While various drugs, such as calcium hydroxide, antibiotics and biocides, are used, no single option is completely effective, and further studies are needed to determine the best treatment for root canal infections.

8. ***Falagas M.E and coworkers (2008)***[17] emphasized that due to the lack of new antibiotics, the medical community has been revisiting the use of older drugs like fosfomycin. A literature review examined 62 studies out of 1311 initially identified, focusing on fosfomycin' s effectiveness and safety in treating gram + and gram - infections (not involving UTIs and gut infections). Among 1604 patients with diverse infections—including pneumonia, meningitis, osteomyelitis, and diabetic foot infections—fosfomycin, used singly or combined with other antibiotics, achieved a remedy in 81.1% of cases and improvement in 2.9%. In surgical prophylaxis studies (mainly colorectal procedures involving 1212 patients), fosfomycin-metronidazole was as effective as other antibiotic combinations. Given its broad-spectrum efficacy, fosfomycin may serve as a viable alternative for treating serious infections, especially amid rising resistance. However, more research is needed to assess its performance against multidrug-resistant organisms like ESBL- and MBL-producing *Enterobacteriaceae* and *Pseudomonas aeruginosa*.

9. ***RJR Delgado and coworkers (2010)***[18] stated that the effectiveness of calcium and hydroxide ion based medicament and 2% chlorhexidine gelatinous form used in eliminating *Enterococcus faecalis* from dentinal tubules during endodontic treatment. Teeth infected with E. faecalis were subjected to treatment with either Ca(OH)2, chlorhexidine gel, a combination of the two, or saline, which served as a control. The findings indicated that both Ca(OH)2 and chlorhexidine substantially decreased bacterial levels and viability when compared to the control group, with chlorhexidine exhibiting greater antimicrobial effectiveness. No additional advantages were found when Ca(OH)2 was used alongside chlorhexidine, suggesting that chlorhexidine alone was more efficient in eradicating E. faecalis.

10. ***Aghmadi F and coworkers*** (2014)[19] said that the most habitable bacteria in a failed endodontic treatment is fund to be Enterococcus faecalis (E. faecalis due to its resistance to calcium hydroxide (CH). This study conducted a systematic review and meta-analysis to evaluate if combining CH with chlorhexidine (CHX) enhances its effectiveness against E. faecalis. A comprehensive search across multiple databases identified 45 relevant studies, with nine included in the meta-analysis. The analysis showed no statistical difference between CH alone and the CH/CHX combination in their antibacterial effect ($p = 0.115$). Current evidence suggests that adding CHX does not improve CH's efficacy against E. faecalis. Additionally in vivo research is needed to confirm these findings in clinical settings

11. ***Kim D. et al and coworkers (2014)***[20] focused on the properties of calcium hydroxide and reviewed findings from in vitro research concerning its antimicrobial activity. The latter part examined in vivo investigations, including both animal experiments and human clinical trials. Animal studies indicated that using calcium based intracanal medicament led to improved histological outcomes. In contrast, clinical trials in humans revealed that while the bacterial load was reduced, complete elimination of microorganisms was not achieved, and certain species

demonstrated resistance to calcium-based medicament. Moreover, various clinical researches concluded that applying $Ca(OH)_2$ between treatment sessions did not enhance the healing of periapical lesions. Ongoing research is necessary to better understand its antimicrobial potential and to identify the most effective materials and techniques for thoroughly disinfecting infected root canals.

12. ***Dijkmans et al and coworkers (2017)***[21] had emphasized on Fosfomycin as a broad-spectrum, bactericidal antibiotic with a low molecular weight, known for its effectiveness against various bacterial strains, including multidrug-resistant Gram-negative organisms. It works by irreversibly blocking the first stage of bacterial cell wall formation. The oral form, fosfomycin tromethamine, has a bioavailability of less than 50%, which is why it can only be used as a single 3-gram dose for uncomplicated urinary tract infections. However, pharmacokinetic and pharmacodynamic studies have explored alternative multi-dose regimens that may support its future use in managing more complex infections caused by resistant bacteria. Despite its potential, the current understanding of Fosfomycin's pharmacology and resistance mechanisms remains incomplete or debated, and therefore, using it alone in treatment is not currently recommended without further clinical evidence.

13. ***Keepers R. et al and coworkers(2017)***[22] conducted this research to measure the antimicrobic activity of fosfomycin in comparison with other antibiotics against selected strains of Enterobacteriaceae isolated from urinary tract infections (UTIs) in the United States. The study examined 658 clinical isolates that were also susceptible to commonly used antibiotics such as ciprofloxacin, levofloxacin, nitrofurantoin, and trimethoprim/sulfamethoxazole. The bacterial strains included *Escherichia coli* (257), *Klebsiella* species (156), *Enterobacter* species (79), *Pseudomonas aeruginosa* (60), *Enterococcus faecalis* (54), and *Proteus* species (52). The minimum inhibitory concentrations for fosfomycin needed to inhibit 50% (MIC_{50}) and 90% (MIC_{90}) of the isolates were found to be 4 µg/mL and 64 µg/mL, respectively. These findings demonstrate

that fosfomycin exhibits substantial in vitro activity against a broad spectrum of UTI pathogens, including antibiotic-resistant strains, and could be considered a viable treatment option for drug-resistant urinary infections.

14. ***Savitha et al and coworkers* (2019)**[23] study conducted in vivo aimed to evaluate the antimicrobial efficacy of 2% chlorhexidine gluconate gel compared to chitosan and a combination of both as treatment for Enterococcus faecalis in teeth that had undergone unsuccessful endodontic procedures. Permanent single-rooted teeth were chosen, and microbiological samples were obtained at three different points. The initial sample (S1) was collected prior to treatment, followed by a second sample (S2) after biomechanical preparation. The teeth were then randomly divided into one of three groups: 2% chlorhexidine gel, 2% chitosan gel, or a blend of the two. After the access cavity was sealed with Fuji IX glass ionomer cement, a final sample (S3) was taken seven days later. RT-PCR (qPCR) was utilized to measure the levels of E. faecalis. The most significant decrease in bacterial count was seen in the group that received the combination of 2% chlorhexidine and chitosan, probably due to the effective delivery of the treatment enabled by the nanoscale dimensions of the chitosan particles.

15. ***Abott et al and coworkers (2020)***[24] explored the effectiveness of oral fosfomycin against *Enterococcus faecalis* and *Enterococcus faecium*, including vancomycin-resistant strains (VRE), due to limited treatment options for enterococcal species. Using a dynamic in vitro infection model and synthetic human urine, 84 isolates were tested for susceptibility, with 16 representative strains evaluated further. *E. faecium* showed lower growth in synthetic urine compared to *E. faecalis*. Fosfomycin's antibacterial activity was assessed under different dosing regimens, simulating both high and low urinary concentrations. *E. faecalis* required higher fosfomycin exposure to achieve a 3-log reduction in bacterial count compared to *E. faecium*. No resistance development was observed after drug exposure. Notably, two low-dose treatments

achieved similar bacterial suppression as a single high-dose treatment, indicating that fosfomycin may be a viable oral option for managing enterococcal UTIs, including those caused by VRE.

16. ***Aguiler M.D and coworkers (2020)***[25] discovered that fosfomycin remained a valuable antibiotic, especially against multidrug-resistant infections, either alone or in combination therapies. Regulatory bodies like the European Medicines Agency and the US FDA are reviewing clinical data to standardize its use across Europe and potentially approve its sale in the US. Fosfomycin's unique mechanism avoids cross-resistance with other antibiotics, though resistance—mainly through enzymatic inactivation via the fosA3 gene on plasmids—is increasingly reported, particularly in Asia among ESBL- and carbapenemase-producing Enterobacterales. While it shows lower activity against *Pseudomonas aeruginosa* than *E. coli*, it is effective in biofilms, especially when used with aminoglycosides. To optimize its clinical use against resistant pathogens, further research is needed, including refining antimicrobial susceptibility testing methods.

17. ***Wangchinda W. et al (2022)***[26] mentioned in his study that the first member of the epoxide class of antibiotics is fosfomycin (FOF). Both parenteral and oral preparations are commercially marketed. While parenteral administration is commonly used for upper urinary tract infections, oral fosfomycin is frequently used to treat women's simple cystitis. With a limited chance of developing resistance to other antimicrobial classes, FOF exhibits broad-spectrum bactericidal action. As a result, parenteral FOF is being utilized more frequently as an adjuvant treatment for extra-urinary tract infections brought on by Gram-negative, multidrug-resistant bacteria.

18. ***Goer. et al and coworkers (2022)***[27] concluded that Fosfomycin is an antibiotic effective against various bacteria to treat uncomplicated urinary tract infections (uUTIs) and various infections. A multicenter study evaluated the performance of the redeveloped fosfomycin gradient diffusion strip (ETEST FO) against reference methods. The study tested *Enterococcus faecalis*, *Staphylococcus* species, and *Enterobacterales*, showing high accuracy, with essential agreement (EA) and categorical agreement (CA) exceeding 90% for most bacteria. While *Enterobacterales* had an 8.8% very major error (VME) rate, MIC values remained within acceptable ranges. The test tended to underestimate MICs for some bacteria and overestimate them for *Klebsiella pneumoniae*, while it was unreliable for *Enterobacter cloacae*.Overall, ETEST FO proved to be an effective and user-friendly alternative to the traditional agar dilution method

19. ***Souza et al and coworkers(2022)***[28] investigated the cytotoxic effects and synergistic antibacterial properties of epigallocatechin gallate (EGCG) and fosfomycin (FOSFO) against biofilms produced by oral bacteria commonly associated with endodontic infections. The minimum inhibitory and bactericidal concentrations (MIC/MBC), as well as the fractional inhibitory concentration (FIC), were assessed for Enterococcus faecalis, Actinomyces israelii, Streptococcus mutans, and Fusobacterium nucleatum. A synergistic effect was noted between EGCG and FOSFO for all species tested, with FIC values between 0.35 and 0.5. None of the treatments demonstrated cytotoxicity to fibroblasts at effective concentrations. The combination of EGCG and FOSFO significantly diminished biofilms of E. faecalis and A. israelii and completely eradicated those of S. mutans and F. nucleatum. Scanning electron microscopy revealed disruption of biofilms and a reduction in the extracellular matrix in multispecies biofilms that were treated with EGCG, the combination, or chlorhexidine (CHX). Confocal imaging indicated a significant reduction in biofilms within the dentinal tubules, with the EGCG+FOSFO combination resulting in the highest percentage of cell death (84.85%). Overall, EGCG and FOSFO exhibited strong synergistic antibacterial effects without exhibiting

cytotoxic properties.

20. ***Isabela N et al and coworkers (2022)*** [29] conducted a study to evaluate how various vehicles used with calcium hydroxide paste influence bacterial reduction in teeth diagnosed with apical periodontitis. Ninety teeth with non vital root canals were tested before treatment (S1) and after biomechanical preparation (S2). The teeth were then grouped into three groups, each receiving a different intracanal medicament for one week: calcium hydroxide in glycerin, camphorated paramonochlorophenol mixed with glycerin (CHPG), or 2% chlorhexidine. A third sample (S3) was collected after the medicament phase. Quantitative PCR (qPCR) was used to assess bacterial load. All groups showed a reduction in bacterial presence after canal preparation. However, the group treated with calcium hydroxide in glycerin showed a slight increase in bacterial counts between S2 and S3, despite a drop in positive cases from 69% to 59%. In the CHPG group, positive cases decreased from 59% to 52%, with a 71% average reduction in bacterial count. The chlorhexidine group saw a decrease from 70% to 57%, with a 55% average reduction. While overall antibacterial performance among the three groups was statistically similar, only the CHPG group demonstrated a significant decrease in bacterial levels between post-preparation and post-medication phases.

21. ***Devi W. et al and coworkers (2022)***[30] suggested how Uncontrolled biofilms are implicated in dental issues such as caries, gingivitis, and periodontitis, with Enterococcus faecalis being a significant contributor to biofilm development. Inhibition of biofilms can be accomplished by targeting the metabolism of the cell wall, interrupting quorum sensing (QS), and reducing virulence factors like gelatinase. The compound 24-propylcholesterol exhibited antibacterial properties against E. faecalis ATCC 29212, showing MIC and MBC values of 78.1 µg/mL and 156.3 µg/mL, respectively. Molecular docking analysis indicated strong binding affinities with important enzymes related to biofilm formation, implying its potential as both an antibacterial and antibiofilm agent. Nevertheless, additional in vitro and in vivo research is necessary to verify its effectiveness.

22. ***Teja et al and coworkers (2023)*** [31] compared the antibacterial effectiveness of calcium hydroxide alone and in combination with various agents against *Enterococcus faecalis*. Four hundred extracted human premolars were injected with *E. faecalis* for 21 days and then divided into five groups based on the disinfecting material used. These included calcium hydroxide alone, and its combinations with chlorhexidine, chitosan, silver nanoparticles, and bioactive glass S53P4. Dentin samples from the apical third were analyzed using culture methods. Statistical analysis revealed that all groups significantly reduced bacterial counts, but the combination of calcium hydroxide with bioactive glass S53P4 showed the highest antibacterial efficacy. The results highlight that combining calcium hydroxide with other agents enhances its antibacterial action, with bioactive glass being the most effective.

23. ***Sunlakawit et al and coworkers (2024)***[32] had studied Calcium hydroxide as a commonly used intracanal medicament, has shown inconclusive effects on the fracture resistance of dentine. This study conducted a systematic review to assess CH's impact on human dentine strength over different timeframes. A comprehensive search of multiple databases up to February 29, 2024, identified 2,265 studies, with 27 meeting the inclusion criteria, covering 3,879 teeth or roots. The analysis confirmed that CH affects dentine fracture resistance, with a significant impact after ≥4 weeks in mature teeth, while no specific duration was identified for primary teeth. The evidence ranged from both the extremes, risk of bias. Short-term CH did not have any effect on dentin, supporting its routine clinical use. While the medicament remains effective for prolonged , risks should be carefully evaluated to ensure tooth preservation and successful treatment outcomes.

24. ***Ahmed S et al and coworkers (2024)*** [33] stated that Endodontic treatment failures are often associated with the presence of *Enterococcus faecalis* and *Candida albicans*. This study systematically evaluated their prevalence in 30 retreatment cases to assess their impact on clinical outcomes. Using standardized clinical, radiographic, and microbiological methods, both

pathogens were frequently detected, showing a strong correlation with treatment failure. The findings highlight the importance of accurately identifying these microorganisms and implementing targeted antimicrobial strategies to enhance the success of endodontic retreatment.

MATERIALS AND METHODS

STUDY SETTINGS

- The study was designed and carried out for Colony Counting laboratory work at the Department of Conservative Dentistry and Endodontics, Santosh Dental College, Ghaziabad, NCR Delhi, in cooperation with the Pioneer Centre o Bio-Sciences for Advanced Training and Research, Mohan Nagar, Ghaziabad.

STUDY DESIGN

- The study was conducted as a double blinded randomized Control Trial. All the PICO guidelines were followed.

- **P (Population/Problem):**

  Patients: Patients requiring nonsurgical endodontic retreatment of single-rooted teeth with persistent periapical lesions

  Condition: Failed root canal therapy due to microbial persistence (specifically *E. faecalis*).

- **I (Intervention):**

  Fosfomycin as an intracanal medicament (gel formulation applied for 7 days).

- **C (Comparison):**

  Calcium hydroxide mixed with 2% chlorhexidine gel (standard intracanal medicament).

- **O (Outcome):**

  Primary: Reduction in colony-forming units (CFUs) of *E. faecalis* post-treatment (quantified via microbiological culture).

- Secondary: Comparative efficacy of fosfomycin vs. $Ca(OH)_2$/CHX.

Key PICO Elements from the Study:

1. **Population**:
    - 60 patients with single-rooted teeth requiring retreatment
    - Confirmed *E. faecalis* via CFU analysis (pre-treatment samples).
2. **Intervention**:
    - **Fosfomycin gel was** prepared at MBC concentration).
3. **Comparison**:
    - **$Ca(OH)_2$ + 2% CHX gel** (control group).
4. **Outcomes**:
    - CFU counts were counted at three stages:
        1. Pre-treatment sample (S1).
        2. Post-biomechanical preparation sample (S2).
        3. Post-medicament application sample (S3).
    - **Statistical significance**: Paired t-test for intragroup reduction; independent t-test for intergroup comparison.

SAMPLE SIZE CALCULATION

60 patients requiring retreatment with periapical radiolucency in single rooted teeth were selected randomly from the Department of Conservative Dentistry and Endodontics, Santosh Dental College, Ghaziabad, NCR Delhi.

ETHICAL CONSIDERATIONS

- The present study was conducted as per the ethical principles laid by screening committee in detail by the Institutional Ethics committee of Santosh Deemed to be University, Ghaziabad, Delhi-NCR. The informed consent was obtained from the patients

CRITERIA FOR CASE SELECTION

Inclusion Criteria –

- Teeth requiring repeat endodntic therapy; characteristic teeth with periapical symptoms
- Root Canal treated teeth showing radiographic evidence of apical pathology
- Teeth with adequate tooth structure for adequate isolation with rubber dam.
- Single rooted Teeth

Exclusion Criteria –

- Medically compromised patients or patients with allergies.
- Periodontal pocket depth of 4 mm or more
- Antibiotics consumption within the past 1 month for any systemic illness
- Teeth having intraradicular posts, vertical root fracture
- Teeth with compromised coronal tooth structure (not restorable)

SAMPLE GROUP

60 patients requiring retreatment in single rooted teeth with periapical lesion were selected. The patients were randomly divided using SPSS software. The SPSS software was used to give random numbers to patients which were further divided into Test Group and Control Group.

- GROUP 1: Control Group ($Ca(OH)_2$ + Chlorhexidine)
- GROUP 2: Test Group (Fosfomycin)

RANDOMIZATION PROTOCOL

A computer-generated randomization list was created using IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp., Armonk, NY, USA). Each of the 60 eligible single-rooted teeth was assigned a unique identification number. A random number was generated for each sample using the RV.UNIFORM(0,1) function in SPSS. The data were then sorted in ascending order based on the random number, and the first 30 cases were allocated to the control group (Calcium Hydroxide + 2% Chlorhexidine), while the remaining 30 were allocated to the test group (Fosfomycin). This ensured unbiased and concealed allocation of samples into study groups.

BLINDING

This study was designed as a double-blind randomized controlled trial. To ensure patient blinding, the two intracanal medicaments were made visually indistinguishable by storing them in opaque bottles. The treatments were applied without revealing which formulation was used, keeping the patients unaware of the study groups.. To ensure operator blinding during microbial analysis, all post-treatment samples were labeled with anonymized codes and sent to the microbiology laboratory, where the evaluator was unaware of the group allocation. Randomization and coding were conducted by a assistant not involved in the treatment or outcome assessment.

ARMAMENTARIUM AND MATERIAL –

GENERAL ARMAMENTARIUM

- Rubber dam (Coltene)
- Kidney tray
- Air rotor (NSK)
- Endo-Z bur (Dentsply)
- Endo Access bur (Dentsply)
- Round burs (SS White)
- K files (Dentsply)
- H files (Dentsply)
- 30 Gauge Needle and Syringe (Dispovan)
- Endomotor (Orikam)
- Vial (4gm) with Reduced Transport Fluid
- Incubator
- Colony Counter
- BHI media
- Distilled Water
- Autoclave
- EDTA 17% (Aveue Prep)
- Sodium Hypochlorite 5%
- Normal Saline (Baxter, Haryana, India)
- Retreatment Files (Dentsply)
- Calcium Hydroxide powder (Prevest)
- Chlorhexidine Gel 2% (Prevest)

- Sodium Thiosulfate (Central Drug House)
- Methyl Cellulose
- Glycerin (Central Drug House)
- Apex Locator (Coltene)
- Temporary Restorative Material (Cavit 3M ESPE)
- Fosfomycin (TCI, Japan)
- Propylene Glycol (Central Drug House)

Fig. 1; Retreatment Files

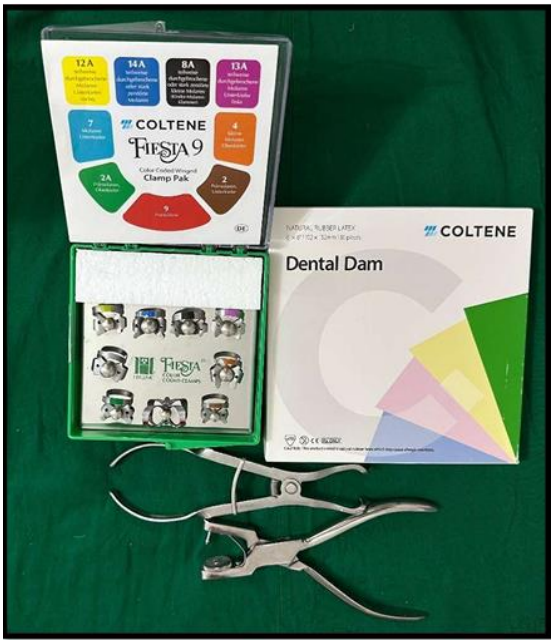


Fig. 2; Rubber Dam

Fig. 3; Airotor/Hand Files

Fig. 4; Apex Locaor

Fig. 5; Calcium Hydroxide Powder

Fig. 6; Chlorhexidine Gel

Fig.7; Gutta Percha

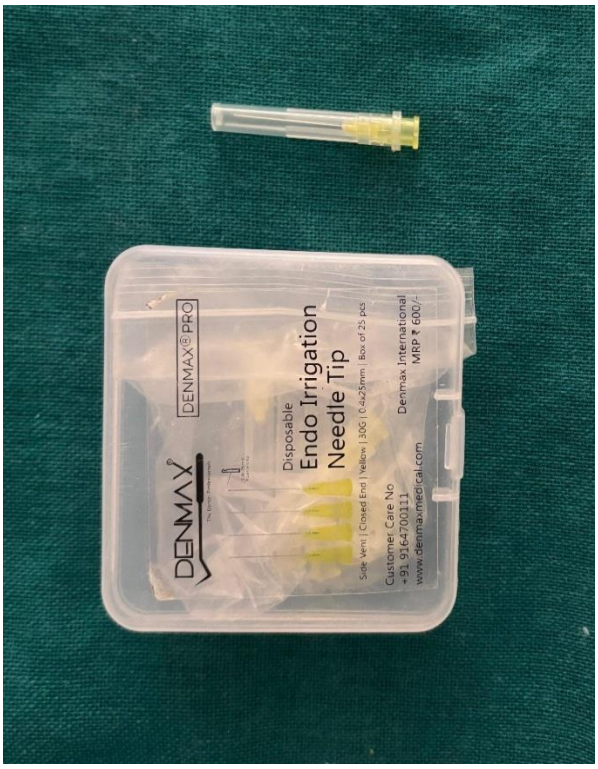


Fig. 8; 30 gauge needles

Fig. 9; S cutting Rotary Files

Fig. 10; Syringes

Fig. 11; Fosfomycin

Fig. 12; Propylene Glycol

CALCULATION OF MINIMUM BACTERICIDAL CONCENTRATION(MBC) OF FOSFOMYCIN AGAINST E. FAECALIS (ATCC 29212)

A broth dilution method is used to calculate Minimum bactericidal - Concentration (MBC) The detection process is as follows:

The bacterial strain was cultured overnight in an incubator to ensure an active growth of E. faecalis. This culture was then diluted to a suitable concentration using Brain Heart Infusion (BHI) broth, which supports the growth of the bacteria. The test compound underwent serial dilution, and an equal volume of the prepared bacterial suspension is added to each dilution. The inoculated samples are incubated under optimal temperature and time conditions. Turbidity in the tubes or wells indicates microbial growth. The Minimum Inhibitory Concentration (MIC) is recognized as the lowest concentration of the test substance at which no visible turbidity is observed, signifying the suppression of bacterial growth. To establish the Minimum Bactericidal Concentration (MBC), the MIC tube along with at least two higher concentrations are inoculated onto agar plates. Following incubation, the number of colony-forming units (CFU/mL) is recorded. The MBC is described as the smallest concentration of the compound that achieves a 99% decrease in microbial viability, thereby indicating bactericidal effectiveness

Fig. 13a; MIC Calculation

Figure 13b; MIC Calculation

Fig. 14; Pipetting

Fig. 15; Streaking

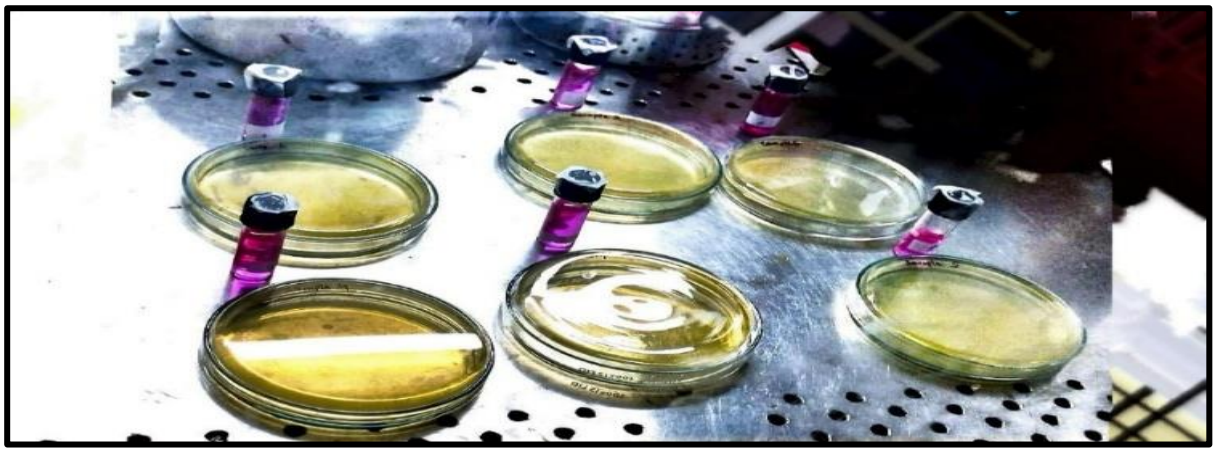


Fig. 16; Sample making

PREPARATION OF MEDICAMENT

PREPARATION OF FOSFOMYCIN MEDICAMENT

Fosfomycin medicament was prepared by adding 1 mL methyl cellulose to 54 µg fosfomycin. The above solution was thoroughly mixed with the micro pipetting technique (Fig. No - 17). The medicament was stirred in a magnetic stirrer for 15 min to achieve an even distribution of the fosfomycin powder in methyl cellulose. The medicament was kept overnight to achieve a gel like consistency.

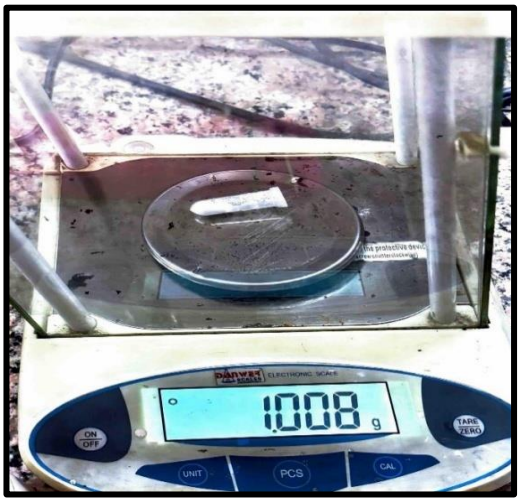


Fig. 17; Weighing of medicament

Fig. 18; Weighing of Methyl Cellulose

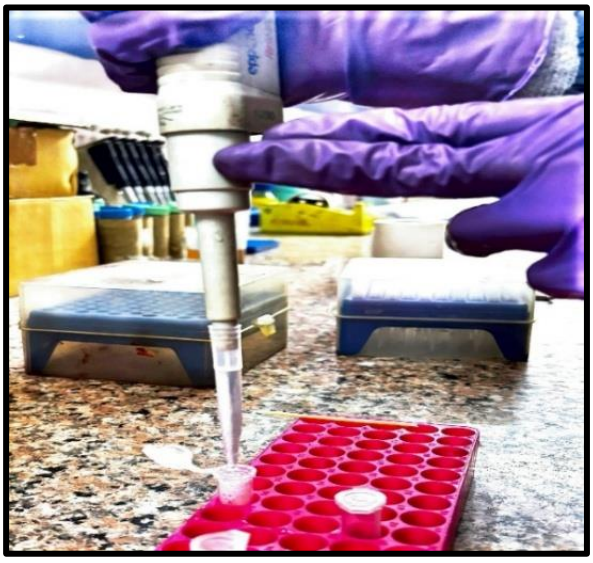


Fig. 19; Micropipetting

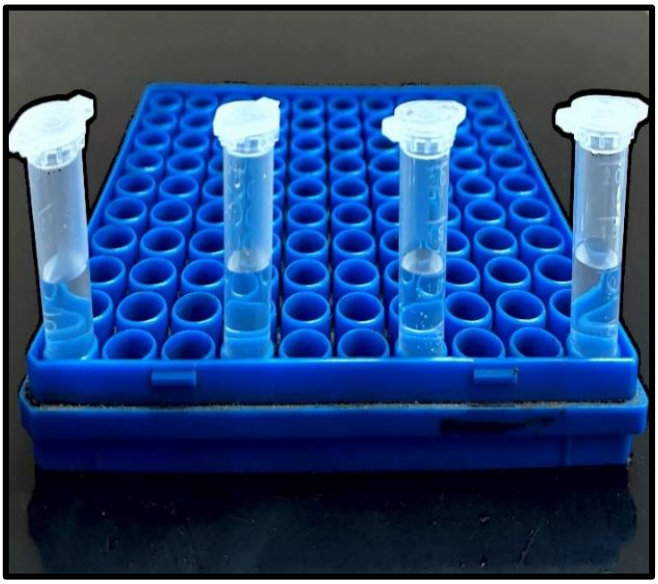


Fig. 20; Medicament Prepared

Fig. 21; Anaerobic Jar

Fig. 22; RTF Vials

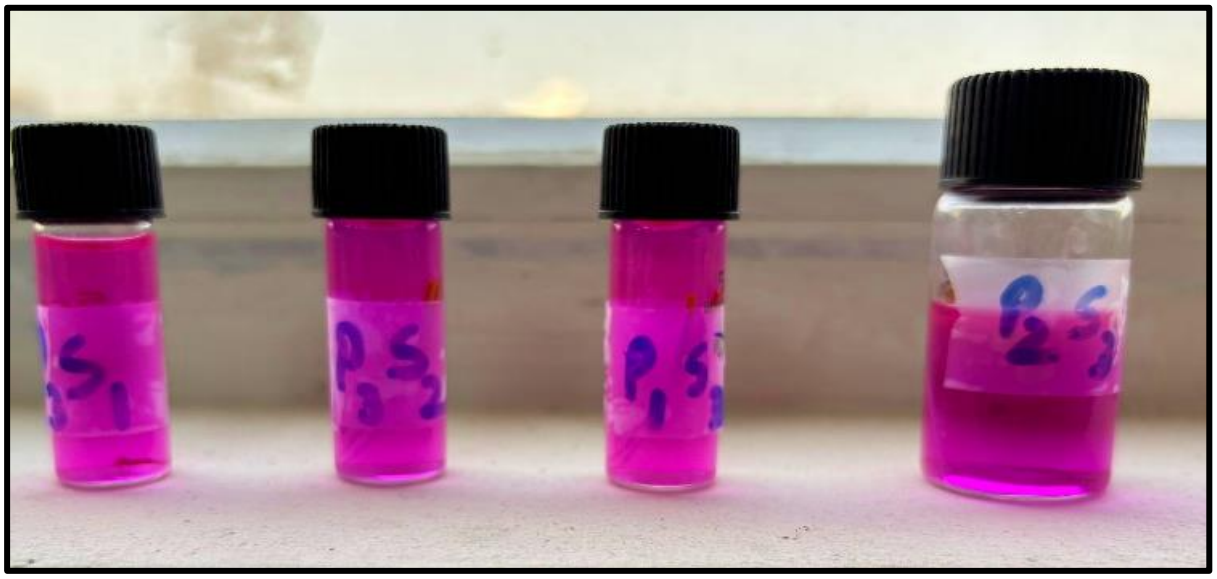


Fig. 23; Sample Collection

PREPARATION OF CALCIUM HYDROXIDE AND CHLORHEXIDINE (2%)

The purest form of 100 mg of Calcium Hydroxide powder was placed on a clean glass slab along with 0.5 mL of Chlorhexidine gel. The powder and gel were mixed using a sterilized stainless-steel spatula to achieve a gel like consistency at room temperature. This medicament was used in our study.

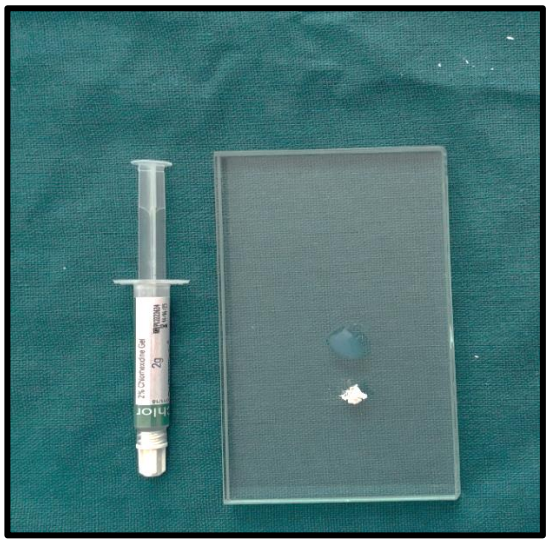


Fig. 24; Calcium Hydroxide powder with Chlorhexidine gel

Fig. 25; Paste consistency of Medicament

METHODOLOGY

The study was conducted at Santosh Dental College and Hospital. 60 patients were selected requiring Retreatment with periapical lesion in single rooted teeth. The inclusion and exclusion criteria was taken into consideration before starting the treatment. Preoperative radiographs were taken to confirm the inclusion criteria

Premedication

A total of 60 patients in need of retreatment for Single Rooted Teeth with periapical lesions were chosen for this study. Aseptic conditions were strictly maintained prior to the initiation of treatment. The tooth and adjacent structures were disinfected using 5% Sodium Thiosulfate, followed by decontamination with 3% Sodium Hypochlorite. To isolate the treated tooth, a Coltene Rubber Dam was used with the appropriate clamp selection. The area of interest was inspected using a sterilized Rhodium Coated mouth mirror. A high-speed handpiece (NSK airotor) equipped with a long-shank round bur or an Endo Access bur (Dentsply) was utilized to remove the post-endodontic filling. After the access cavity was created, it was swabbed with 5% Sodium Thiosulfate, which neutralizes Sodium Hypochlorite without affecting the bacterial sampling.

Once the root canal orifices were identified, the Gutta Percha was extracted by engaging the filling material with H files or Protaper Re-Treatment files (Dentsply) and employing a pulling technique to retrieve it. The dentinal debris, along with the extracted Gutta Percha, was placed in a sterile tube containing 0.75 ml of Reduced Transport Fluid (RTF), constituting Sample 1. The tooth was assessed after the Gutta Percha removal, and a radiographic examination was performed to verify the complete extraction of the Gutta Percha. Measurements of the working length were taken using a K file with a 2% taper (Mani) and an Apex locator (J Moreta). A thorough irrigation process followed, utilizing 3 ml of sterile saline administered via a sterile syringe fitted with a 30 gauge side vented needle. Two paper points were used to absorb the fluid in the canal, which were then placed inside the canal for a minute before being transferred to a sterile tube containing 0.75 ml RTF, along with the remnants of GP from Sample 1.

Next, the root canal was shaped to a size of 25 6% with extensive irrigation, commencing with 17 4% rotary files (Dentsply) while upholding the sterilization protocol. Sodium Hypochlorite was the first irrigant introduced into the root canal, followed by its neutralization with 5% Sodium Thiosulfate. Throughout the procedure, there was continual irrigation with saline. After performing BMP, the canal was dried with paper

points, resulting in Sample 2. Once this sample was collected, the canal was thoroughly dried using the last employed rotary paper points. An intracanal medicament was then applied inside the canal using a Lentulospiral up to the determined working length, and the cavity was sealed with a temporary restorative material. The patient was scheduled for a follow-up visit after 7 days.

The collected samples, Sample 1 and Sample 2, were sent to Pioneer Microbiological Center for testing to detect the presence of E. faecalis. The sample fluid was inoculated onto Brain Heart Infusion media via streaking and incubated for a period of 72 hours. After this incubation period, the plates were removed, and the count of E. faecalis colonies was obtained using the Colony Forming Unit method.

Post medication

Appropriate isolation and disinfection procedures were implemented, specifically involving the placement of a rubber dam and the sterilization of the tooth and its surrounding area with 5% Sodium Thiosulfate. The Cavit was removed using a round bur attached to a high-speed handpiece that had a continuous water supply. The root canal was flushed with Normal Saline through a 30-gauge side-vented needle. The canal was thoroughly rinsed to eliminate the intracanal medicament. Paper points were used to dry the canal, and these points were then placed into the RTF vial. Sample 3 will be collected. Sample 3 was obtained during the second appointment. These samples were sent to Pioneer Microbiological Center for analysis to check for the presence of E.faecalis. The sample fluid was applied to Brain Heart Infusion media using the streaking method and incubated for 72 hours. After 72 hours, the plates were removed from the incubator to assess the growth of E.faecalis bacteria, and colony counting was done using Colony Forming Unit.

TEST GROUP/ PATIENT 1
(Fosfomycin)

S. No	Pt. Name	Age	Tooth No.
1	Sheetal	27 years	45

Fig 26a; Preoperative Sample

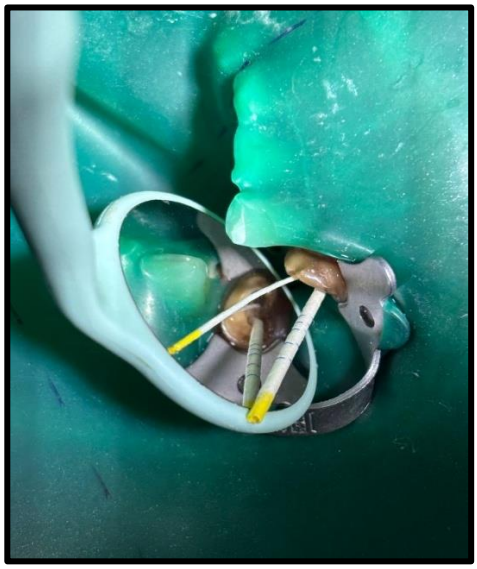


Fig. 26b; Post BMP Sample

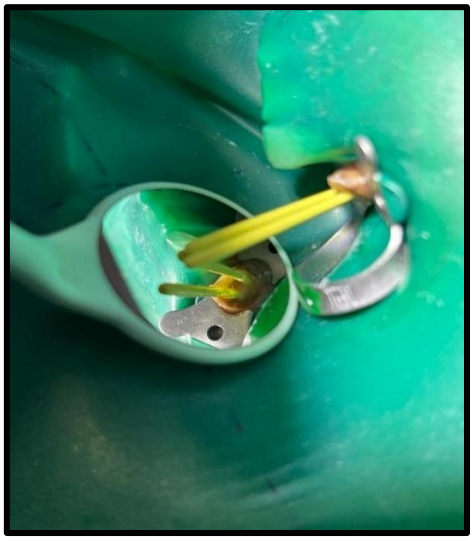


Fig. 26c; Post Medicament Sample

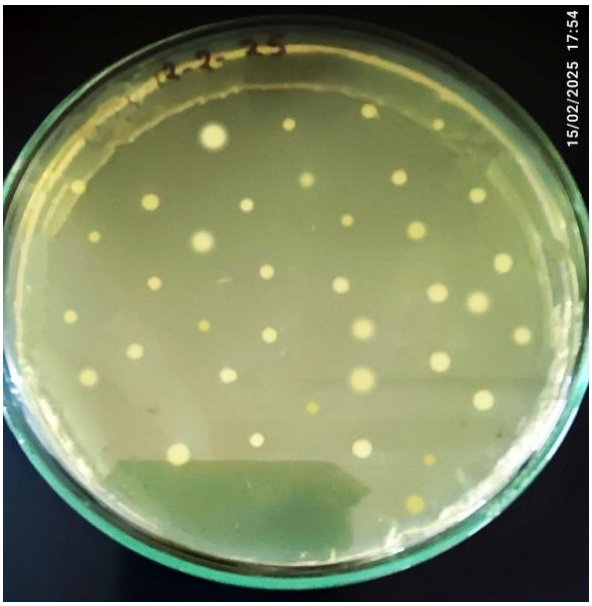


Fig 26d; Microbial Culture (S1)

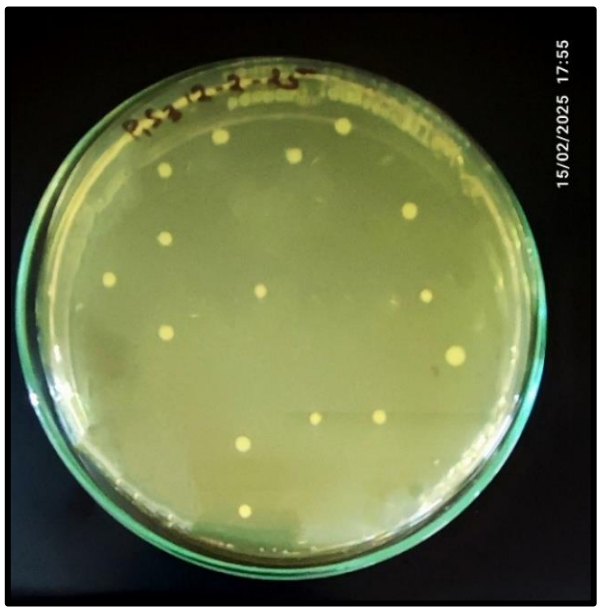


Fig. 26e; Microbial Culture (S2)

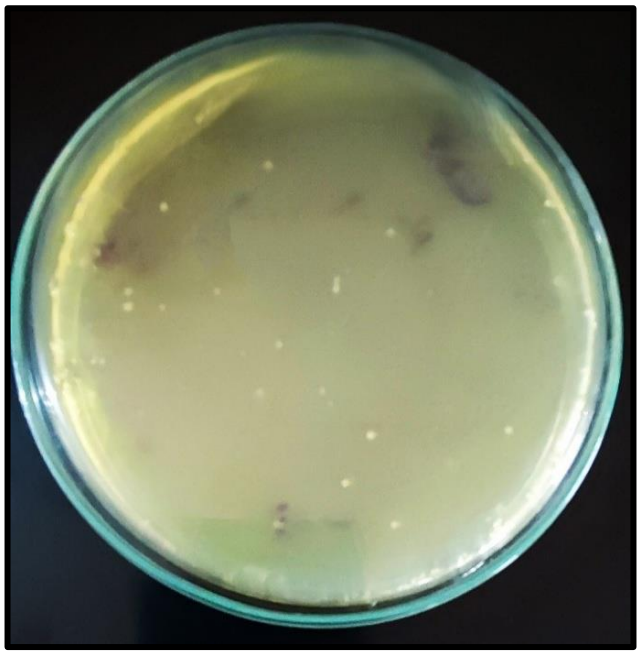


Fig 26f; Microbial Culture (S3)

TEST GROUP/ PATIENT 2

S. No	Pt. Name	Age	Tooth No.
2.	Surbhi	32 years	25

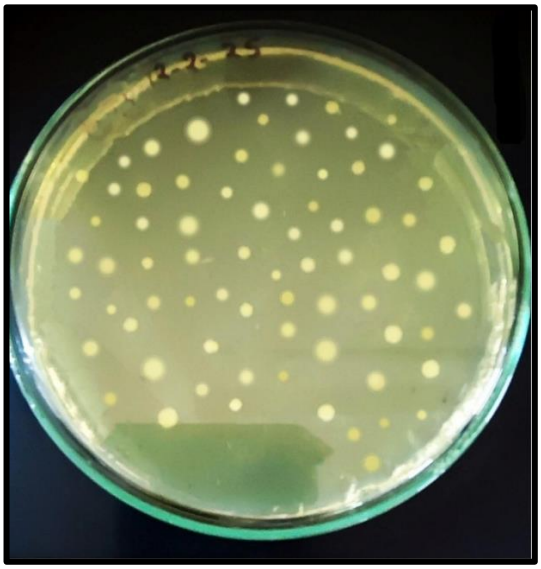


Fig 27a; Microbial Culture (S1)

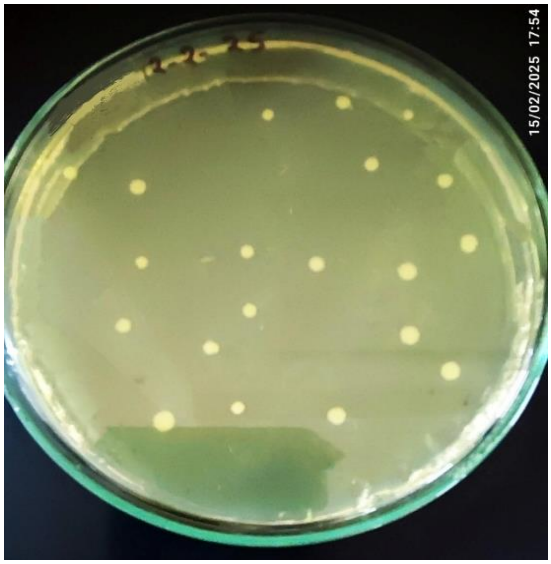


Fig 27b; Microbial Culture (S2)

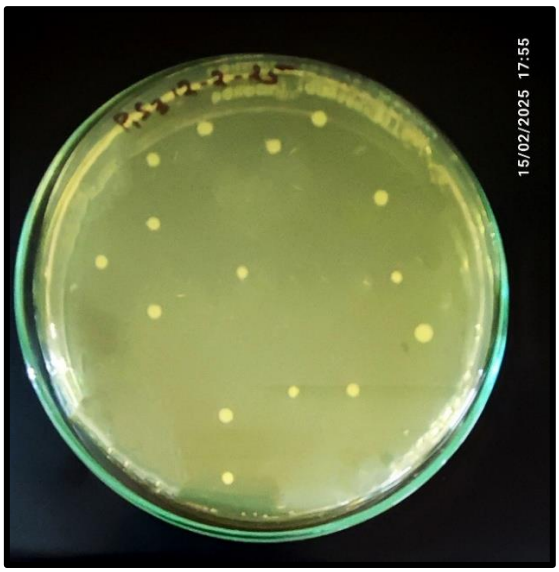


Fig 27c; Microbial Culture (S3)

CONTROL GROUP/ PATIENT 1

(Calcium Hydroxide +2 % chlorhexidine Gel)

S. No	Pt. Name	Age	Tooth No.
3.	Rajesh	45 years	21,22,23

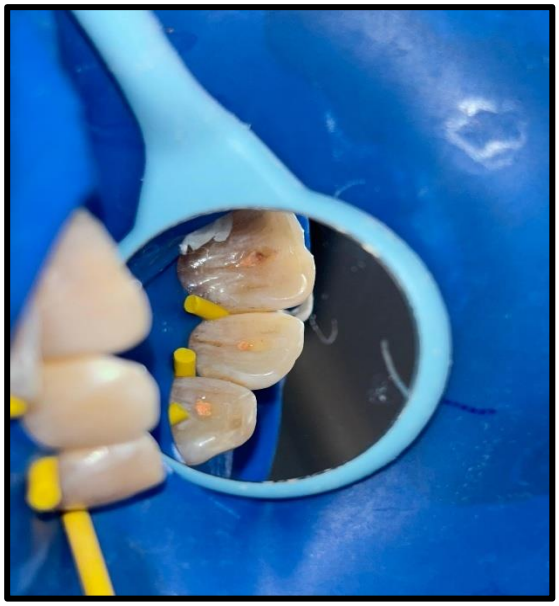


Fig 28a; Preoperative clinical

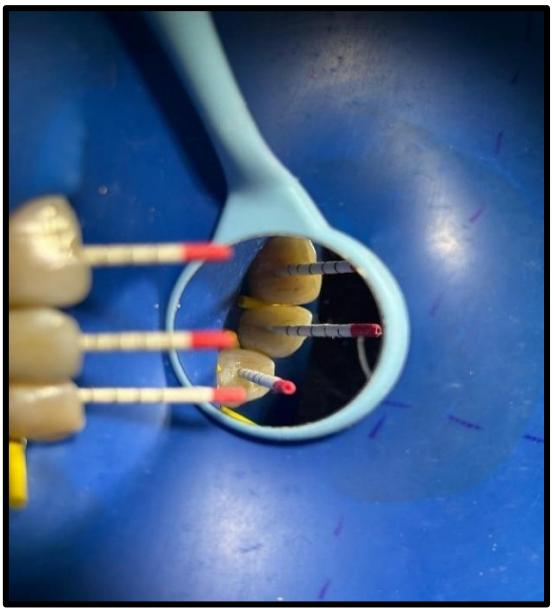


Fig 28b; Post BMP (S2)

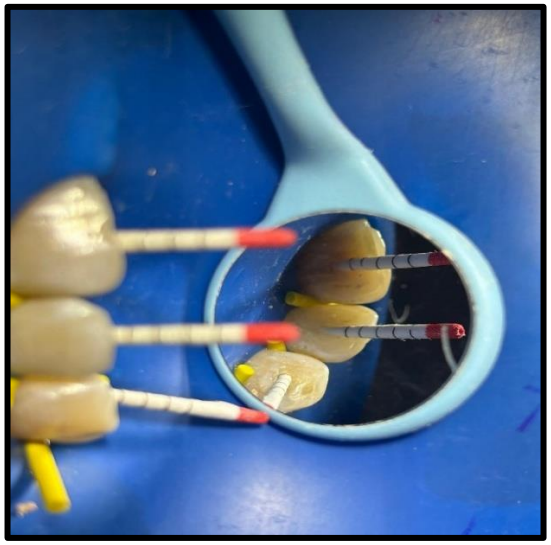


Fig. 28c; Post Medicament Sample (S3)

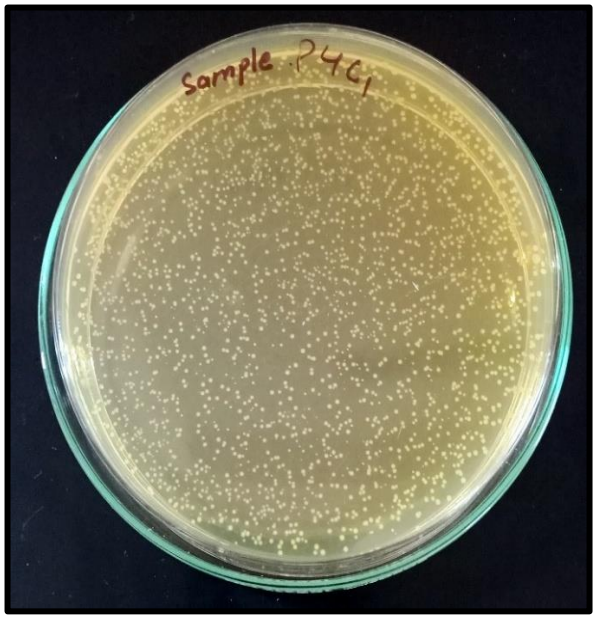


Fig 28d; Microbial Culture (S1)

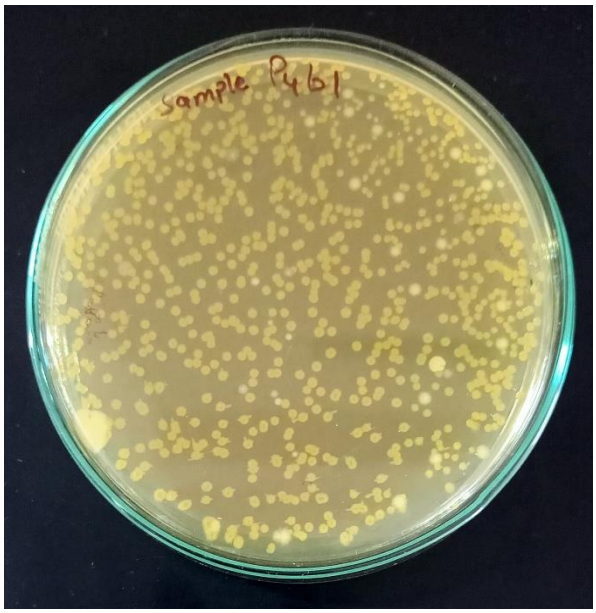


Fig 28e; Microbial Culture (S2)

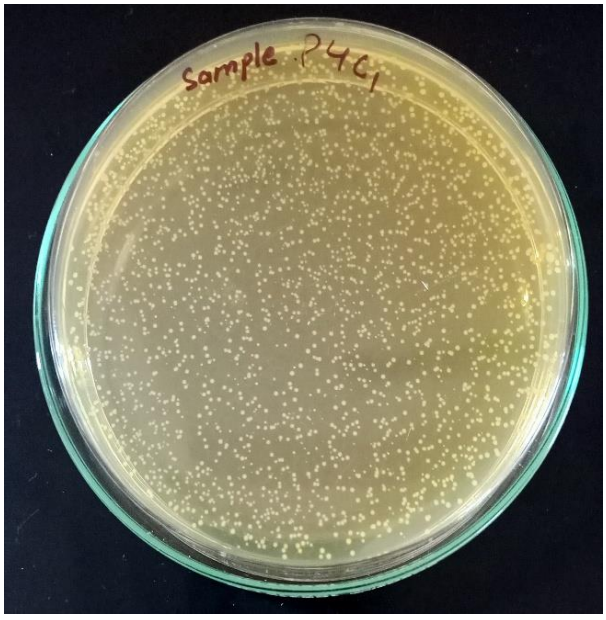


Fig 28f; Microbial Culture (S3)

CONTROL GROUP/ PATIENT 2

S. No	Pt. Name	Age	Tooth No.
4.	**Arpit**	**23 years**	**22**

Fig 29a; Preoperative

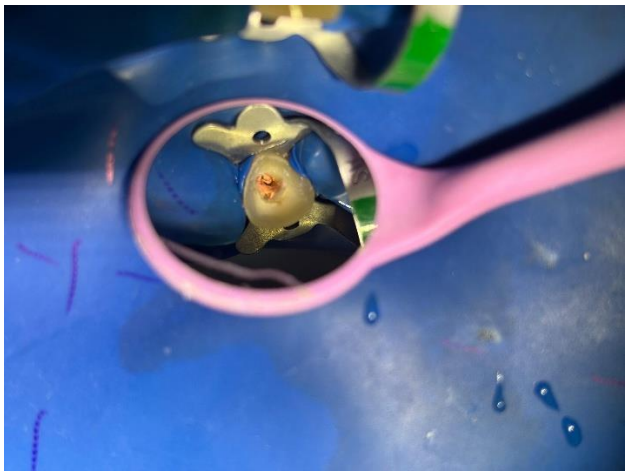


Fig 29b; Access Cavity

Fig 29c; Post GP retreival

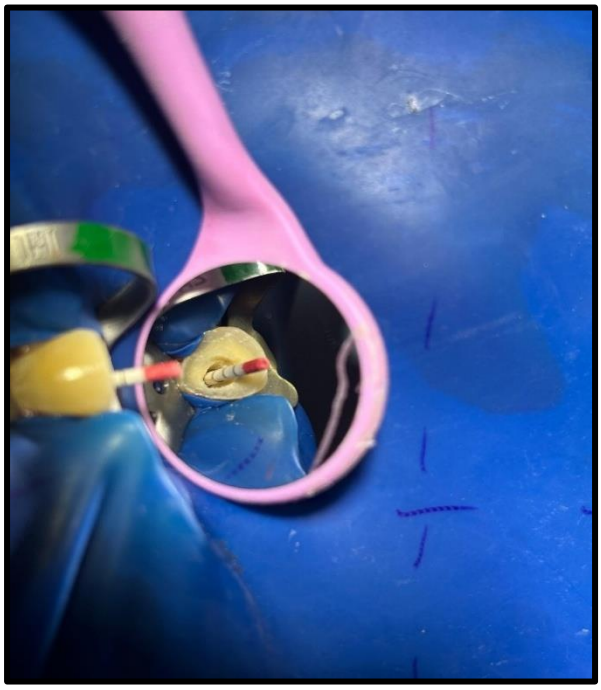


Fig. 29d; Preoperative Sample

Fig. 29e; Post BMP Sample

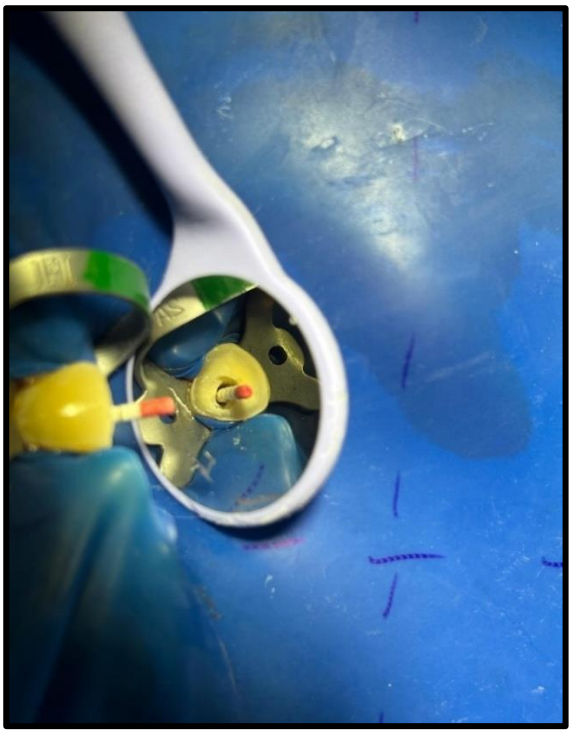


Fig 29f; Post Medicament Sample

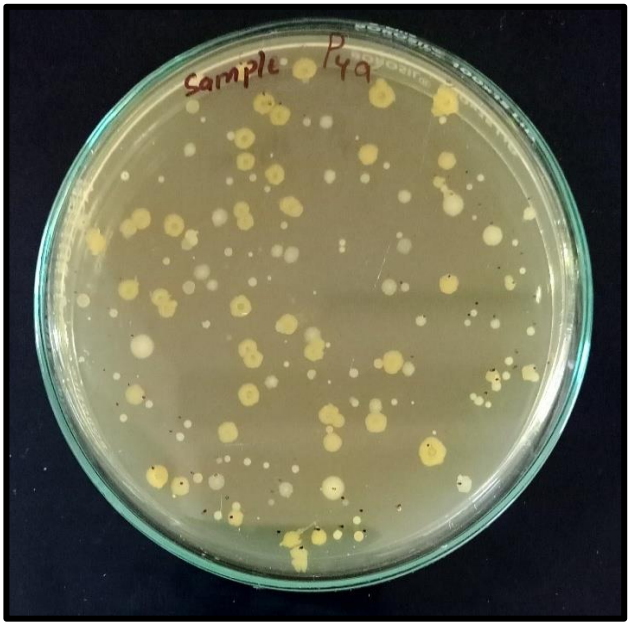


Fig. 29g; Microbial Culture (S1**)**

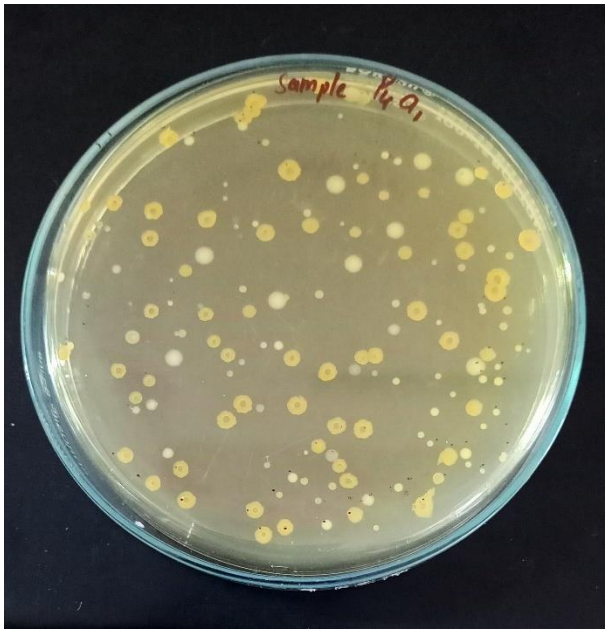


Fig 29h; Microbial Culture (S2)

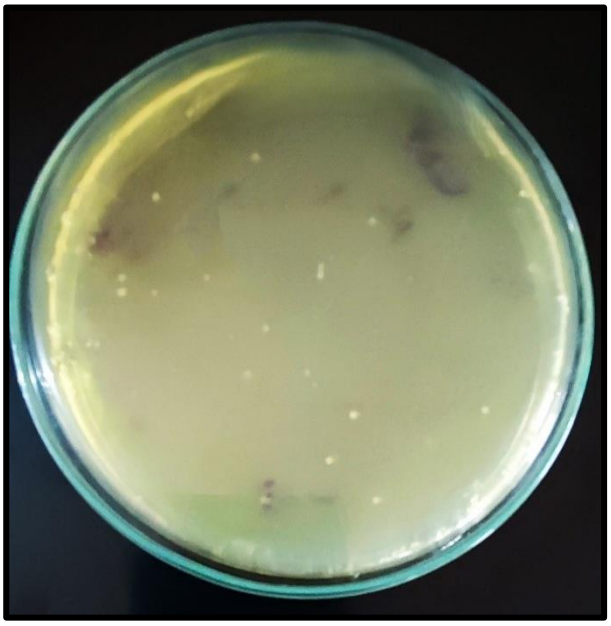


Fig. 29i; Microbial Culture (S3)

STATISTICAL ANALYSIS

- Descriptive data will be expressed as number/ percentage and frequency and quantitative data will be expressed as Mean ± SD or Median, Interquartile range.

- Normality assessment will be done using Kolmogorov- Smirnoff test. •

- Test for comparative assessment analysis will be decided based on findings of normality assessment.

STATISTICAL ANALYSIS

Microsoft Excel 2007 facilitated the data entry for this study, while SPSS statistical software, version 23.0, was employed for the analysis. Descriptive statistics included the computation of the mean, standard deviation, frequency, and percentage. In this study, a significance level of 5% was established. The independent t-test was utilized for comparing different groups, whereas the paired t-test was conducted for comparisons within the same group over time. The distribution of the data was assessed using the Shapiro-Wilk test, and the homogeneity of the variables was evaluated through Levene's test.

❖ **Mean**

$$\overline{X} = \frac{\Sigma X}{N}$$

- Where:
  - $\overline{X}$ = the data set mean
  - $\sum$ = the sum of
  - X = the scores in the distribution
  - N = the number of scores in the distribution

❖ **Range**

$$range = X_{highest} - X_{lowest}$$

- Where:

  $X_{highest}$ = largest score

  X_{lowest} = smallest score

❖ **Variance**

$$SD^2 = \frac{\Sigma(X - \overline{X})^2}{N}$$

The simplified variance formula

$$SD^2 = \frac{\Sigma X^2 - \frac{(\Sigma X)^2}{N}}{N}$$

- Where:

  SD^2 = the variance

  $\sum$ = the sum of

  X = the obtained score

  $\overline{X}$ = the mean score of the data

  N = the number of scores

❖ **Standard Deviation (N)**

$$SD = \sqrt{\frac{\Sigma(X - \overline{X})^2}{N}}$$

The simplified standard deviation formula

$$SD = \sqrt{\frac{\Sigma X^2 - \frac{(\Sigma X)^2}{N}}{N}}$$

Where:

SD = the standard deviation

$\sum$ = the sum of

X = the obtained score

$\overline{X}$ = the mean score of the data

N = the number of scores

❖ **Independent t-test**

Independent t Test can be used to if there is an evident difference between two sets of data,and is most commonly applied when the test statistic would follow a normal distribution. The independent samples *t*-test is used when two separate sets of independent and identically distributed samples are obtained, one from each of the two populations being compared

$$t = \frac{\overline{X}_1 - \overline{X}_2}{\sqrt{\left(\frac{(N_1 - 1)s_1^2 + (N_2 - 1)s_2^2}{N_1 + N_2 - 2}\right)\left(\frac{1}{N_1} + \frac{1}{N_2}\right)}}$$

Where X1 =Mean of the first Group, X2 =Mean of the Second Group

❖ **Paired t test**

$$t = \frac{\bar{x} - 0}{SE(d)} = \frac{\bar{x}}{SD(x)/\sqrt{n}}$$

A paired t-test is a statistical method which can be used to compare the means of two groups somewhere related to each other. This test is appropriate when each value in one sample has a corresponding or "paired" value in the other sample. Common scenarios include evaluating outcomes before and after an intervention on the same subjects—such as assessing students' performance before and after a training program—or comparing two different techniques or treatments applied to the same individuals or units. The paired nature of the data helps to control for individual variability, making the comparison more accurate.

RESULTS

30 patients from were selected for Control and Test group. The preoperative, intraoperative and post operative samples were sent to Pioneer Labs and the colonies were counted using a Colony Forming Unit. The E.Faecalis was

CONTROL GROUP MASTER CHART -

`	SAMPLE 1	SAMPLE 2	SAMPLE 3
1.	70	35	20
2.	153	107	76
3.	96	65	83
4.	59	67	48
5.	85	39	45
6.	235	168	107
7.	129	73	62
8.	>300	234	157
9.	68	42	26
10.	59	33	19
11.	103	82	54
12.	196	95	25
13.	45	86	59
14.	154	103	74
15.	129	87	58
16.	153	124	92
17.	98	75	46
18.	102	81	57
19.	197	156	107
20.	164	132	96
21.	90	48	32
22.	128	85	47
23.	243	179	102
24.	110	68	76
25.	>300	256	165

26.	119	147	106
27.	139	103	66
28.	190	153	132
29.	87	64	43
30.	65	38	74

Table 1

TEST GROUP MASTER CHART

S.NO	SAMPLE 1	SAMPLE 2	SAMPLE 3
1.	129	94	36
2.	253	198	103
3.	>300	>300	247
4.	221	164	86
5.	157	110	58
6.	196	124	65
7.	128	147	126
8.	186	135	142
9.	167	108	102
10.	141	130	113
11.	139	65	25
12.	182	117	63
13.	126	113	56
14.	249	169	120
15.	194	135	94
16.	171	151	105
17.	156	129	65
18.	>300	278	203
19.	217	163	114
20.	126	110	60
21.	76	54	23
22.	48	35	15
23.	163	110	86
24.	96	82	39
25.	247	127	61
26.	26	12	5
27.	143	114	19
28.	229	182	105
29.	117	96	31
30.	79	45	15

Table 2

NORMALITY TEST TO CHECK FOR THE NORMALITY OF THE DATA

GP		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Pre	Control Group	.157	30	0.058	.868	30	0.055
	Test Group	.081	30	0.200*	.984	30	0.910
During Treatment	Control Group	.163	30	0.080	.898	30	0.087
	Test Group	.145	30	0.105	.926	30	0.089
After Treatment	Control Group	.122	30	0.200*	.940	30	0.090
	Test Group	.136	30	0.161	.909	30	0.084

Table 3

The normality tests for both the **Control** and **Test** groups across the three time points (**Pretreatment**, **During Treatment**, and **After Treatment**) indicate that the data largely follows a normal distribution.

In the Control Group, the results of the Kolmogorov-Smirnov test indicate that there are no significant departures from normality at any of the time points, as the p-values exceed 0.05, suggesting that the data adheres to a normal distribution. Likewise, the Shapiro-Wilk test reflects borderline normality, with p-values nearing but just above 0.05, implying that the data is roughly normally distributed.

The Test Group also displays normality at all time points. The Kolmogorov-Smirnov and Shapiro-Wilk tests indicate no significant departures from a normal distribution for both the Pre-treatment and During Treatment phases, with p-values significantly exceeding 0.05.

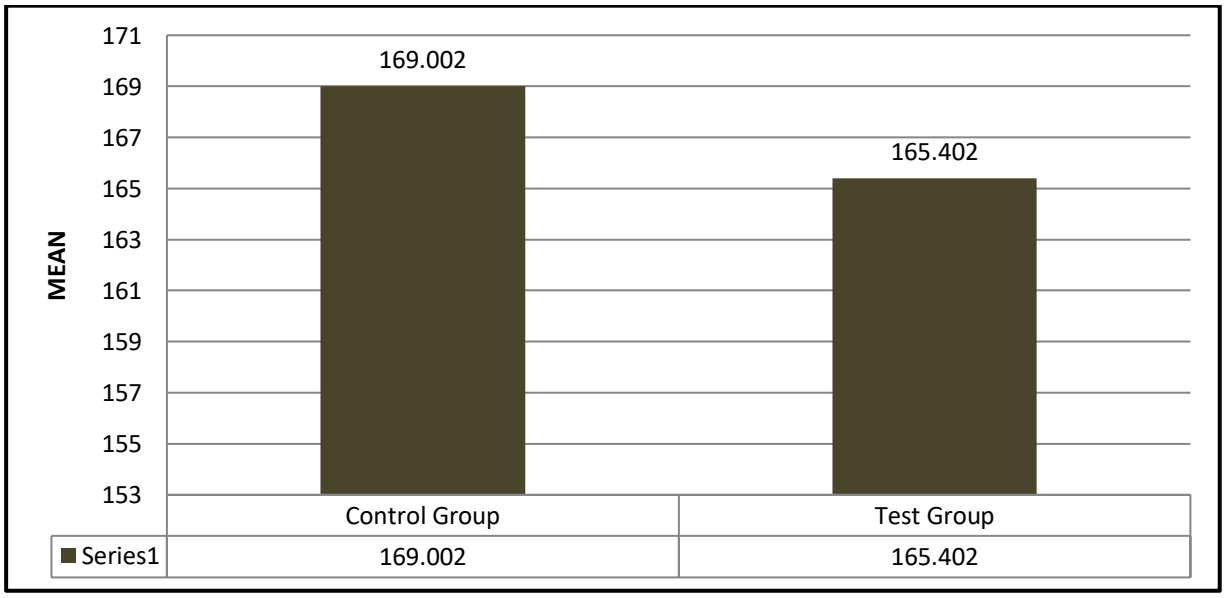
INTERGROUP COMPARIOSN OF MEAN CFU OF E FACEALIS BETWEEN THE GROUPS

	ı	Std. Deviation	Std. Error Mean	P value	Significance
Control Group	002	6	2		Non-Significant
Test Group	402	8	5		

(Control Group = Calcium Hydroxide + Chlorhexidine (2%), Test Group = Fosfomycin)

Table 4

The comparative analysis of the average colony-forming units (CFU) of Enterococcus faecalis between the Control and Test groups shows no statistically significant difference in bacterial proliferation. The Control group, which was administered a blend of calcium hydroxide and 2% chlorhexidine, has an average CFU of 169.002, accompanied by a standard deviation of 77.516 and a standard error of the mean (SEM) of 14.152. In contrast, the Test group, treated with fosfomycin, has an average CFU of 165.402, with a standard deviation of 67.838 and a SEM of 12.385. The P value is 0.838, exceeding the conventional significance level of 0.05, signifying that there is no notable difference between the two groups regarding the reduction of Enterococcus faecalis. This implies that both the combination of calcium hydroxide and chlorhexidine and fosfomycin demonstrate similar effectiveness in diminishing the presence of Enterococcus faecalis in this investigation.



Graph 1

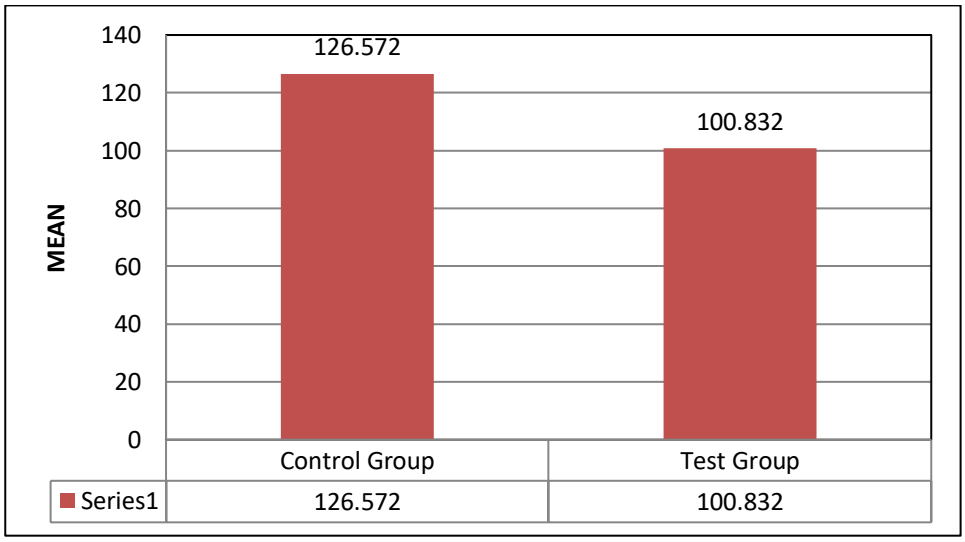
INTERGROUP COMPARIOSN OF MEAN CFU OF E FACEALIS BETWEEN THE GROUPS DURING THE TREATMENT

		Std. Deviation	Std. Error Mean	P value	Significance
Control Group	72	3	9		Non-Significant
Test Group	32	5	6		

(Control Group = Calcium Hydroxide + Chlorhexidine (2%), Test Group = Fosfomycin)

Table 5

The comparison of the average colony-forming units (CFU) of Enterococcus faecalis during the treatment indicates that there is no statistically significant difference between the Control and Test groups. In the Control group, which was treated with calcium hydroxide and 2% chlorhexidine, the mean CFU is 126.572, accompanied by a standard deviation of 61.503 and a standard error of the mean (SEM) of 11.229. The Test group, which received fosfomycin, recorded a mean CFU of 100.832, with a standard deviation of 56.505 and a SEM of 10.316. The P value obtained is 0.165, exceeding the significance level of 0.05, thus signifying that there is no statistically significant difference in the reduction of Enterococcus faecalis between the two groups during treatment.



Graph 2

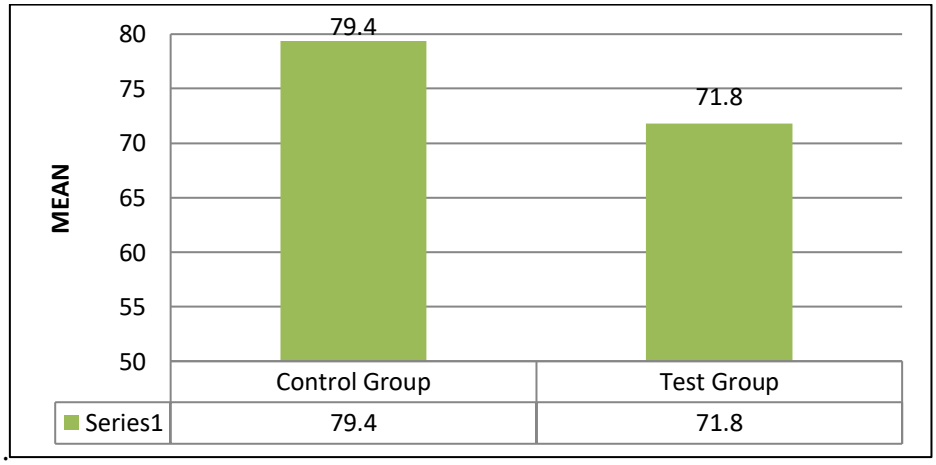
INTERGROUP COMPARISON OF MEAN CFU OF E FAECALIS BETWEEN THE GROUPS AFTER THE TREATMENT

	Mean	Std. Deviation	Std. Error Mean	P value	Significance
Control Group	79.400	55.083	10.056	0.078	Non-Significant
Test Group	71.800	37.623	6.869		

(Control Group = Calcium Hydroxide + Chlorhexidine (2%), Test Group = Fosfomycin)

Table 6

The comparison of mean colony-forming units (CFU) of Enterococcus faecalis between the Control and Test groups after treatment reveals no significant statistical difference. The Control group, which received calcium hydroxide and 2% chlorhexidine, had a mean CFU of 79.400, accompanied by a standard deviation of 55.083 and a standard error of the mean (SEM) of 10.056. Conversely, the Test group, treated with fosfomycin, exhibited a mean CFU of 71.800, with a standard deviation of 37.623 and a SEM of 6.869. The P value obtained is 0.078, slightly exceeding the typical significance level of 0.05. This suggests that the disparity between the groups is not statistically significant, indicating that both treatments (calcium hydroxide + chlorhexidine and fosfomycin) demonstrate similar effectiveness in reducing Enterococcus faecalis after treatment



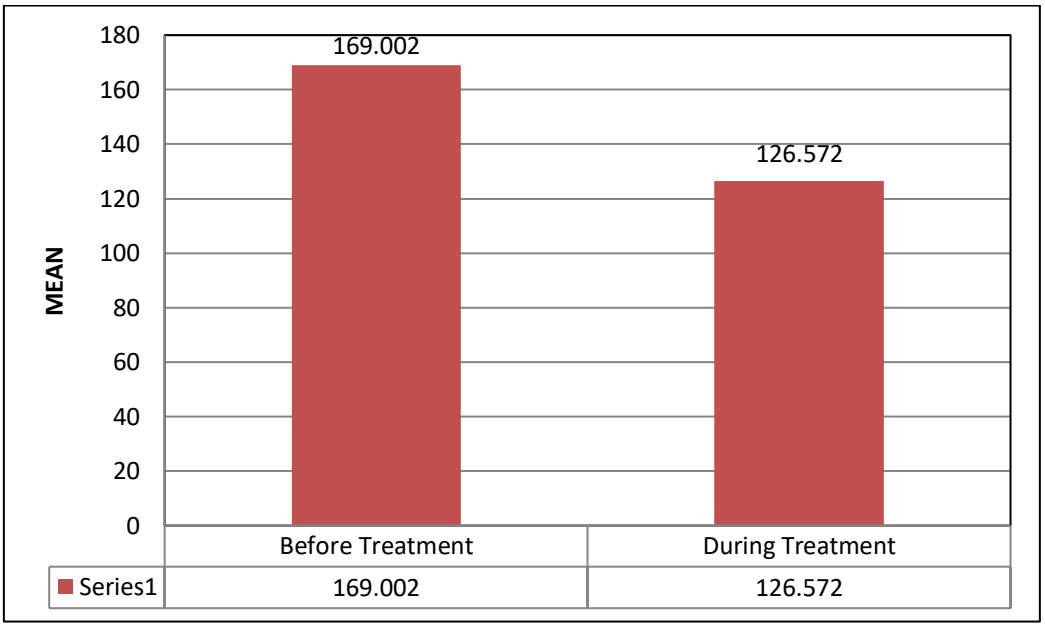
Graph 3

INTRAGROUP COMPARISON OF MEAN CFU OF E FAECALIS BETWEEN DIFFERENT TIME INTERVALS IN CONTROL GROUP

Group	Time Point	Mean ± SD	Mean Reduction	P Value	Significance
Control Group	Before Treatment	169.002 ± 77.516	42.430±16.11	**0.001**	**Significant**
	During Treatment	126.572 ± 61.503			

Table 7

In the **Control Group**, there was a statistically significant reduction in the mean colony-forming units (CFU) of *Enterococcus faecalis* following treatment. The mean CFU before treatment was **169.002 ± 77.516**, which decreased to **126.572 ± 61.503** during treatment. This corresponds to a **mean reduction of 42.430 ± 16.11 CFU**, with a **P value of 0.001**, indicating that the observed difference is statistically significant.



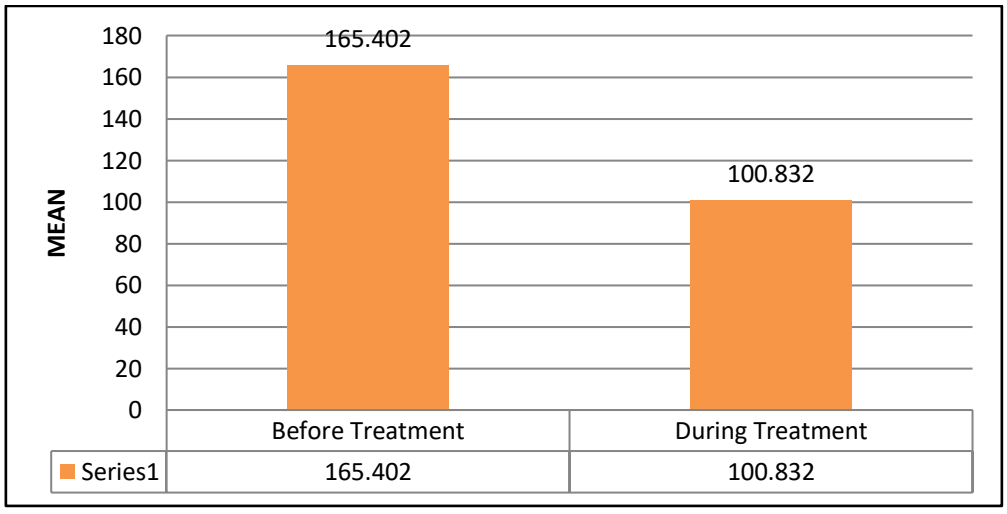
Graph 4

INTRAGROUP COMPARISON OF MEAN CFU OF E FAECALIS BETWEEN DIFFERENT TIME INTERVALS IN TEST GROUP

Group	Time Point	Mean ± SD	Mean Reduction	P Value	Significance
Test Group	Before Treatment	165.402 ± 67.838	64.570±11.76	**0.001**	**Significant**
	During Treatment	100.832 ± 56.505			

Table 8

In the **Test Group**, a significant reduction in the mean colony-forming units (CFU) of *Enterococcus faecalis* was observed between the two time intervals. The mean CFU count before treatment was **165.402 ± 67.838**, which decreased to **100.832 ± 56.505** during treatment. This reflects a **mean reduction of 64.570 ± 11.76 CFU**, with a **P value of 0.001**, indicating a statistically significant decrease. These results demonstrate that the treatment administered in the test group was effective in substantially reducing the bacterial load of *E. faecalis* over the treatment period.



Graph 5

INTRAGROUP COMPARISON OF MEAN CFU OF E FAECALIS BETWEEN DIFFERENT TIME INTERVALS IN TEST AND CONTROL GROUP

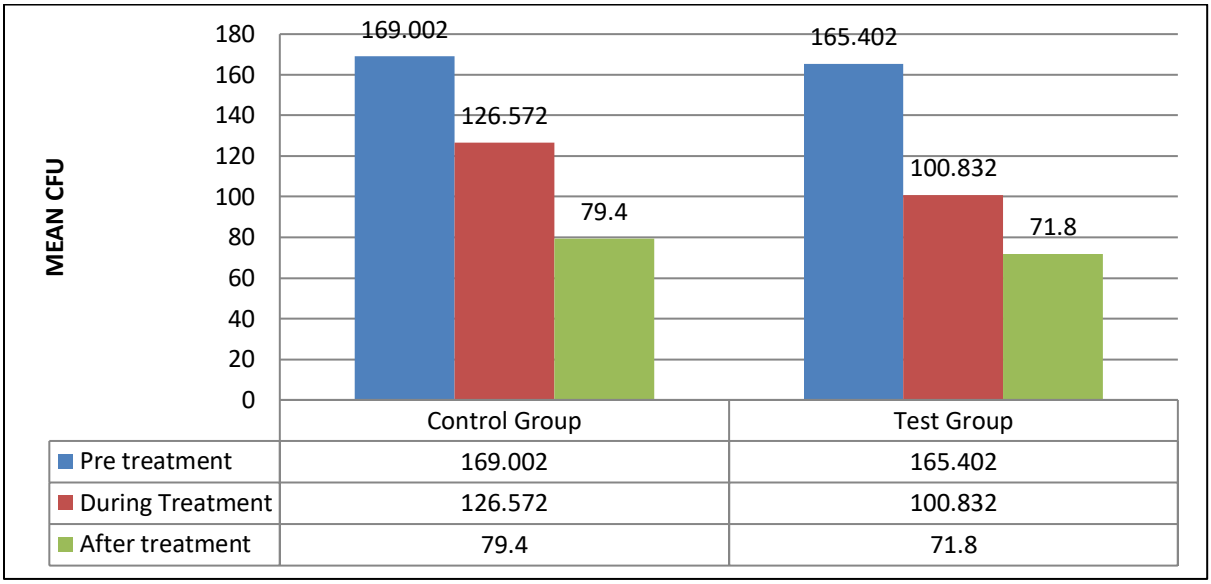
Group	Time Point	Mean ± SD	P Value	Significance
Control Group	Before Treatment	169.002 ± 77.516	**0.00**1	**Significant**
	During Treatment	126.572 ± 61.503		
	After Treatment	79.400 ± 55.083		
Test Group	Before Treatment	165.402 ± 67.838	**0.00**1	**Significant**
	During Treatment	100.832 ± 56.505		
	After Treatment	71.800 ± 37.623		

Table 9

The intragroup comparison of the mean colony-forming units (CFU) of *Enterococcus faecalis* between different time intervals in both the Control and Test groups shows a significant reduction in bacterial growth over time. In the **Control group**, which received calcium hydroxide and 2% chlorhexidine, the mean CFU decreased from **169.002 ± 77.516** before treatment to **126.572 ± 61.503** during treatment, and further to **79.400 ± 55.083** after treatment, with a significant P value of **0.001**. This indicates that the treatment effectively reduced *Enterococcus faecalis* over time.

Similarly, in the **Test group**, which was treated with fosfomycin, the mean CFU decreased from **165.402 ± 67.838** before treatment to **100.832 ± 56.505** during treatment, and further to **71.800 ± 37.623** after treatment, also with a significant P value of **0.001**. This suggests that fosfomycin was similarly effective in reducing *Enterococcus faecalis*.

Overall, both the Control and Test groups demonstrated a significant reduction in bacterial growth, indicating that both calcium hydroxide + chlorhexidine and fosfomycin are effective treatments in reducing *Enterococcus faecalis* during the course of the study.



Graph 6

The results of this randomized controlled trial demonstrated that both calcium hydroxide combined with 2% chlorhexidine (control group) and fosfomycin (test group) were effective in significantly reducing *Enterococcus faecalis* colony-forming units (CFUs) in retreatment cases of single-rooted teeth. Intragroup comparisons showed a statistically significant reduction in bacterial load at each stage—after biomechanical preparation and following intracanal medicament application—within both groups ($p < 0.001$). Although the fosfomycin group exhibited lower mean CFU values at all time points compared to the control group, intergroup comparisons revealed that these differences weren't statistically significant ($p > 0.05$). The data interpreted as eventhough fosfomycin may have a slightly enhanced antimicrobial effect, both medicaments performed comparably in reducing *E. faecalis* under the conditions of this study

DISCUSSION

The bacterial load teeth undergoing retreatment with periapical radiolucency have been examined in a number of studies. Regarding the main species in instances with failed endodontic treatment, studies produced varying findings. According to Pinheiro[34], in cases that had already been treated,, E. faecalis dominated with the maximum bacterial count when examined through various culture methods in the tooth (45.8%). Sedgley et al.[35] also confirmed the results of the previous. Furthermore, in contrast to other studies, the low percentages of E. faecalis demonstrated that the microbe is not the only primary cause of endodontic failure.

Various studies were held to examine the mechanism of survival of E.faecalis under severe surrounding conditions after multiple dressings with carious medicaments[36] E. faecalis can create biofilms to shield itself against disinfectants and host resistance, and it can also use the fluid in the periodontal ligament for nutrition. In addition to those methods, E. faecalis's capacity to penetrate dentinal tubules and attach itself to collagen, as well as to develop antibiotic resistance, particularly to various antibiotics[37]

Key virulence factors of *Enterococcus faecalis* include aggregation substances, surface adhesins, lipoteichoic acid, extracellular superoxide, and the lytic enzymes gelatinase and hyaluronidase. Aggregation substances, which are plasmid-encoded, aid in plasmid transfer during bacterial conjugation and enhance bacterial attachment to eukaryotic cells and collagen types I and IV. They also help *E. faecalis* evade neutrophil responses. These substances can trigger immune reactions, such as the release of TNF-α from macrophages and INF-γ and TNF-β from T cells stimulated by the bacteria.the release of Tumor Necrosis Factor contributes to bone resorption, while INF-γ promotes the production of hydrogen peroxide and superoxide anions, leading to cellular and tissue damage. Additionally, surface adhesins enhance *E. faecalis* virulence by enabling it to attach to various surfaces—including biofilm-

forming abiotic materials, other bacteria (for gene and nutrient exchange), collagen, human serum, and dentin[37]. Only one study examines *E. faecalis* survival under specific conditions.

Ran S et al. investigated how the bacterium adapts to alkaline stress, specifically at pH 10. They found that *E. faecalis* alters the expression of 613 genes. The up-regulated genes were mainly involved in amino acid and nucleotide transport and metabolism, indicating the bacterium's ability to use certain amino acids for energy and pyrimidine synthesis in alkaline environments, thereby enhancing its virulence. In contrast, the down-regulated genes were linked to carbohydrate and amino acid metabolism[38].

In alkaline environments, the glycolytic pathway in *E. faecalis* is disrupted, prompting the bacteria to rely on alternative metabolic routes that utilize less favorable energy sources like fructose and mannose. Alongside gene regulation changes, *E. faecalis* also produces stress-related proteins to cope with environmental stress. However, blocking protein synthesis with chloramphenicol does not affect bacterial survival, suggesting these stress proteins play a minimal role in enduring high pH levels[40].

A key survival mechanism is the proton pump, which helps maintain internal pH balance. When exposed to highly alkaline conditions, such as those caused by calcium hydroxide, the proton pump actively transports protons into the cell to reduce cytoplasmic alkalinity. This system functions effectively until it reaches saturation at pH 11.5, beyond which it fails, leading to bacterial cell death [41]

Calcium hydroxide (CH) is one of the widely used interappointment medicament used in the field of endodontics. As a strong base, it interacts with aqueous fluids and releases calcium and hydroxyl ions through a dissociation reaction. These highly reactive ions exert antimicrobial effects by interacting aggressively with various biomolecules at the application site [42]. The hydroxyl ions released from calcium hydroxide exert antibacterial effects by diffusing throughout the root canal system, either through direct or indirect contact [42]. Although CH has long been regarded as the gold standard intracanal medicament, its

effectiveness against *Enterococcus* species—especially when these bacteria are embedded within dentinal tubules—remains uncertain. The antibacterial effects of calcium hydroxide ($Ca(OH)_2$) are primarily due to its high pH, which disrupts bacterial cytoplasmic membranes, denatures proteins, and damages DNA [43,44].

Chlorhexidine gluconate is also used as an irrigating solution and an interappointment

medicament because of its enhanced compatibility with the soft tissues, long-lasting bactericidal/bacteriostatic action (substantivity), and broad-spectrum efficacy. Its antimicrobial activity stems from its positively charged (cationic) molecules, which bind to the negatively charged bacterial cell membranes, causing leakage of the cell's internal contents [45]. CHX is effective against all gram staining bacteria and has proven efficacy even against microorganisms that show resistance to calcium hydroxide. It acts by penetrating bacterial cells and disrupting membrane charge balance[46]. Both agents kill bacteria by compromising the integrity of their cell membranes. Furthermore, in alkaline conditions, chlorhexidine may facilitate the production of reactive oxygen species, which can further inhibit E. faecalis by causing damage to the cell wall and plasma membrane through destruction mediated by nitric oxide[47]. A study by Delgado et al. indicated that Calcium Hydroxide combined with Chlorhexidine was more effective than using Calcium Hydroxide alone.[48]

Fosfomycin is a bactericidal, broad-spectrum antibiotic with a low molecular weight, effective against a range of bacteria, including multidrug-resistant Gram-negative strains [49]. It works by irreversibly blocking an early step in bacterial cell wall synthesis. Studies indicate that Fosfomycin can act synergistically when used with other antibiotics, targeting different bacterial processes, reduced quantity and minimizing toxicity. It does not bind to plasma proteins and is primarily excreted through the kidneys [50].

Limitations of Calcium Hydroxide

Calcium hydroxide is one of the most common interappointment medicament since decades

as it has a high Ph which neutralize the acidic toxins released by bacteria[51]. However, multiple studies highlight its limitations:

- **Reduced Effectiveness Against Resistant Bacteria:**

Enterococcus faecalis, often found in persistent root canal infections, can survive in high pH environments and resist calcium hydroxide treatment. A systematic review concluded that $Ca(OH)_2$ was not consistently effective in eliminating *E. faecalis* from root canals[52].

- **Limited Antimicrobial Activity in Clinical Conditions:**

Although in vitro studies show bactericidal effects, clinical studies report incomplete bacterial elimination, particularly in previously treated teeth[53]

- **Negative Impact on Dentin Properties:**

Continued practice of calcium hydroxide has shown to weaken the structural integrity of dentin, potentially compromising the tooth's strength [54]

2. Advantages of Fosfomycin

Fosfomycin, a broad-spectrum antibiotic, is gaining attention in endodontics due to its unique properties:

- **Effective Against Resistant Pathogens:**

Fosfomycin disrupts bacterial cell wall synthesis and shows efficacy against both Gram + and Gram - bacteria, including antibiotic-resistant strains like *E. faecalis*. It is particularly effective where calcium hydroxide fails [55]

- **Biofilm Penetration:**

Studies indicate fosfomycin can penetrate bacterial biofilms more effectively than many traditional agents, allowing better eradication of persistent infections [56]

- **Synergistic Use:**

When used in combination with other agents, fosfomycin has shown synergistic effects, enhanced its antimicrobial power and broadened its activity spectrum

The findings of this study offer important information regarding the antimicrobial effectiveness of fosfomycin compared to the traditional combination of calcium hydroxide and chlorhexidine in dealing with Enterococcus faecalis during endodontic retreatment procedures. These results highlight the ongoing difficulty presented by E. faecalis, owing to its capacity to adjust and endure in challenging environments, along with its resistance to frequently used intracanal treatments.

While calcium hydroxide combined with chlorhexidine has shown significant antimicrobial action, consistent with existing literature, it is evident that complete eradication of *E. faecalis* remains difficult[57]. The observed antimicrobial effect can be credited to the increased pH of calcium hydroxide, which interferes with bacterial metabolism, and the broad-spectrum action and substantivity of chlorhexidine[58]. However, limitations such as buffering by dentin and bacterial resistance mechanisms may reduce their long-term effectiveness in clinical scenarios.

Fosfomycin, on the other hand, demonstrated promising antimicrobial performance in this study. Its ability to inhibit early stages of bacterial cell wall synthesis and its potential to penetrate dentinal tubules more effectively due to its low molecular weight may contribute to its superior or comparable results. Given its distinct mechanism of action and reduced likelihood of cross-resistance with other endodontic agents, fosfomycin represents a promising alternative in the context of persistent infections, especially in retreatment cases where standard therapies may fail.

The reduction in CFU counts observed with fosfomycin highlights its potential to serve as

an effective intracanal medicament, either as a standalone agent or in combination with other materials. However, while the current study provides encouraging preliminary data, further research is necessary to establish optimal concentrations, exposure times, and biocompatibility in vivo. Long-term clinical studies and investigations into its effect on biofilm disruption and periapical healing are also warranted.

In conclusion, the findings from this randomized controlled trial suggest that fosfomycin may offer comparable, if not superior, antimicrobial efficacy to the traditional calcium hydroxide–chlorhexidine combination against *Enterococcus faecalis*. Incorporating such novel agents into endodontic protocols may enhance treatment outcomes, particularly in retreatment cases where microbial resistance is a significant concern. Continued exploration and innovation in intracanal disinfection strategies are essential for improving success rate

CONCLUSION

Root canal retreatment poses a considerable challenge in the field of endodontics, mainly due to the ongoing presence of microbial infections, particularly Enterococcus faecalis. This microorganism is infamous for its ability to endure extreme environmental conditions, infiltrate deep into dentinal tubules, and resist many standard intracanal medicaments. Consequently, the removal of E. faecalis from the root canal system, especially in cases of retreatment, is a primary objective for achieving successful endodontic results.

In this randomized controlled study, two intracanal medicaments—fosfomycin and a combination of calcium hydroxide with 2% chlorhexidine—were compared for their antimicrobial efficacy against *E. faecalis* in single-rooted teeth requiring retreatment. The primary parameter used to evaluate effectiveness was the reduction in bacterial colonies, measured through colony-forming unit (CFU) counts at three stages: before treatment, after biomechanical preparation, and 7 days after application of the medicament.

The results demonstrated that both medicaments were capable of significantly reducing bacterial load within their respective groups. This reduction was statistically significant over time, indicating that each medicament played an effective role in lowering *E. faecalis* levels. However, when comparing the two groups directly, the difference in CFU reduction between the fosfomycin and calcium hydroxide with 2% chlorhexidine gel groups was not statistically significant.

Although fosfomycin showed a slightly greater reduction in CFUs, the absence of statistical significance may be attributed to several factors. The sample size, while appropriate for a preliminary evaluation, may not have been large enough to detect smaller intergroup differences. Moreover, variability in host response, anatomical differences between teeth, and microbial virulence factors could have influenced the results. Nonetheless, the numerical trend in favour of fosfomycin supports further investigation.

Fosfomycin, a broad-spectrum antibiotic, functions by inhibiting bacterial cell wall synthesis at an early stage. Its ability to act on resistant strains, penetrate biofilms, and maintain efficacy in the presence of dentinal fluid makes it an attractive candidate for intracanal use. On the other hand, calcium hydroxide, despite its long-standing use in endodontics, has shown limited action against *E. faecalis*, especially due to the bacterium's ability to survive in high pH environments using proton pumps and biofilm defenses. The addition of chlorhexidine enhances calcium hydroxide's efficacy by introducing a substantivity component and disrupting bacterial membranes, but limitations remain, particularly against resistant strains embedded in dentin.

One of the notable strengths of this study is the methodological rigor in sample collection, bacterial culturing, and CFU quantification. The use of standardized protocols and statistical validation enhances the reliability of the findings. Fosfomycin's minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were calculated and used to prepare the medicament in a gel form, ensuring precise and clinically relevant dosing.

Although the intergroup comparison did not yield statistical significance, the consistent reduction in bacterial load within both groups indicates that both medicaments are effective. Fosfomycin, due to its pharmacological properties and favourable results in this study, emerges as a potential alternative to conventional agents, especially in retreatment scenarios involving resistant pathogens.

Clinical Relevance and Future Recommendations

The findings of this study opens the door to the possible integration of fosfomycin into endodontic disinfection protocols. While traditional medicaments such as calcium hydroxide and chlorhexidine continue to offer valuable antimicrobial activity, their limitations—especially against persistent organisms like *E. faecalis*—necessitate the exploration of more effective alternatives. Fosfomycin demonstrates promising attributes in this regard and may enhance disinfection outcomes in complex or refractory cases.

However, it is important to note that this study was conducted under controlled clinical conditions with a limited sample size. To validate these findings, larger-scale clinical trials with longer follow-up periods are needed. Additional studies should also explore:

- The efficacy of fosfomycin in teeth with complex canal anatomy.
- Its potential synergistic use with other medicaments or irrigants.
- Its long-term effect on periapical healing and reinfection prevention.
- Biocompatibility and potential effects on dentin and periapical tissues.
-

Final Thoughts

In conclusion, this study demonstrated that both calcium hydroxide with 2% chlorhexidine gel and fosfomycin was effective in reducing *E. faecalis* in endodontic retreatment cases. Although the difference in efficacy was not statistically significant, fosfomycin showed a favourable trend and may serve as a novel and promising intracanal medicament. Its application in retreatment protocols, particularly against resistant pathogens, should be further explored through advanced research. The evolving understanding of antimicrobial resistance and biofilm behaviour highlights the need for continued innovation in intracanal disinfection strategies. Fosfomycin could play a pivotal role in that evolution.

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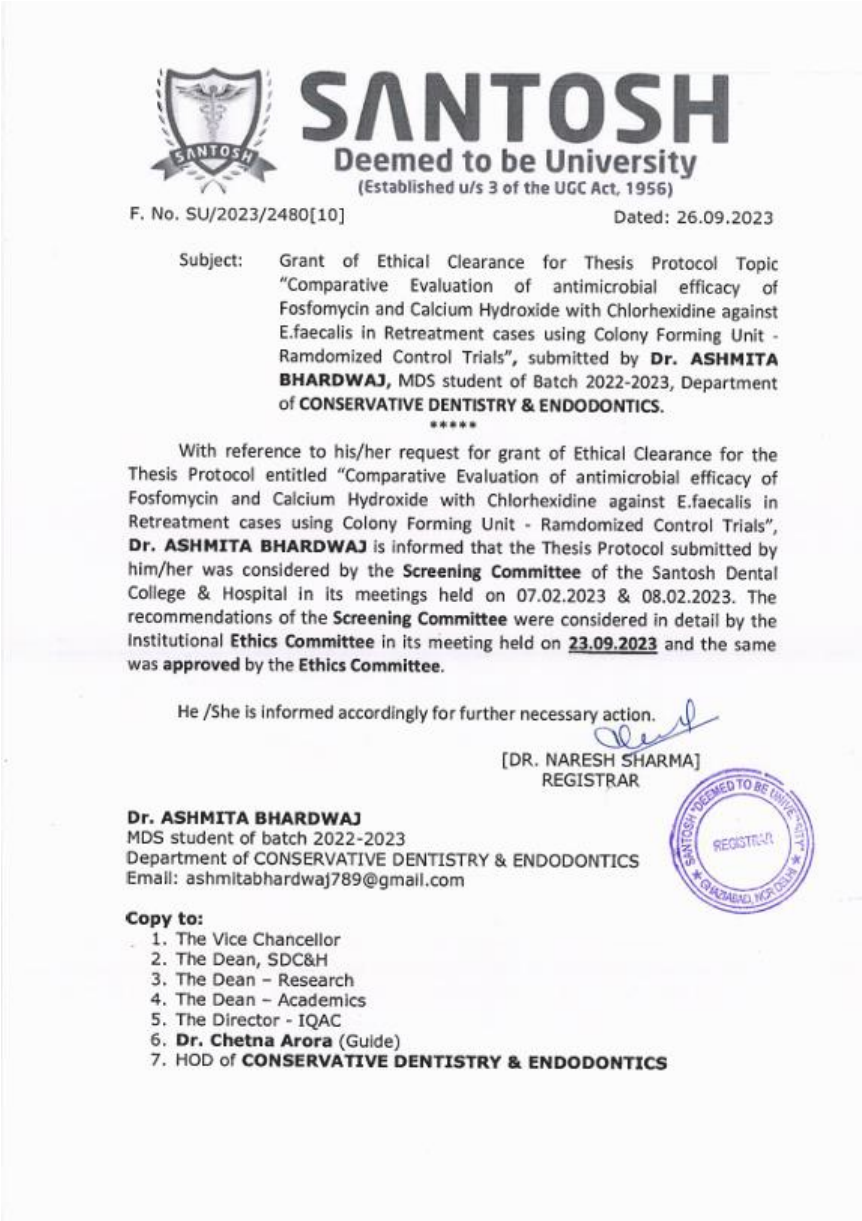
ANNEXURES

ANNEXURE 1
ETHICAL COMMITTEE APPROVAL FORM



SANTOSH
Deemed to be University
(Established u/s 3 of the UGC Act, 1956)

F. No. SU/2023/2480[10] Dated: 26.09.2023

Subject: Grant of Ethical Clearance for Thesis Protocol Topic "Comparative Evaluation of antimicrobial efficacy of Fosfomycin and Calcium Hydroxide with Chlorhexidine against E.faecalis in Retreatment cases using Colony Forming Unit - Ramdomized Control Trials", submitted by **Dr. ASHMITA BHARDWAJ,** MDS student of Batch 2022-2023, Department of **CONSERVATIVE DENTISTRY & ENDODONTICS.**

*****

With reference to his/her request for grant of Ethical Clearance for the Thesis Protocol entitled "Comparative Evaluation of antimicrobial efficacy of Fosfomycin and Calcium Hydroxide with Chlorhexidine against E.faecalis in Retreatment cases using Colony Forming Unit - Ramdomized Control Trials", **Dr. ASHMITA BHARDWAJ** is informed that the Thesis Protocol submitted by him/her was considered by the **Screening Committee** of the Santosh Dental College & Hospital in its meetings held on 07.02.2023 & 08.02.2023. The recommendations of the **Screening Committee** were considered in detail by the Institutional **Ethics Committee** in its meeting held on **23.09.2023** and the same was **approved** by the **Ethics Committee.**

He /She is informed accordingly for further necessary action.

[DR. NARESH SHARMA]
REGISTRAR

Dr. ASHMITA BHARDWAJ
MDS student of batch 2022-2023
Department of CONSERVATIVE DENTISTRY & ENDODONTICS
Email: ashmitabhardwaj789@gmail.com

Copy to:
1. The Vice Chancellor
2. The Dean, SDC&H
3. The Dean – Research
4. The Dean – Academics
5. The Director - IQAC
6. **Dr. Chetna Arora** (Guide)
7. HOD of **CONSERVATIVE DENTISTRY & ENDODONTICS**

ANNEXURE II
CONSENT FORM (ENGLISH)

Department of Conservative Dentistry and Endodontics

INFORMED CONSENT FORM

1. I _____,age_____ son/daughter of _____R/O_____gives full consent for the examination, investigation and the treatment, without any compulsion of any kind.

2. Doctor has fully explained to me the purpose of the procedure(s), the predictable and unpredictable consequences of the procedure, drug administered if any including the use of local anaesthesia , and has also informed me of expected benefits and complications (from known and unknown causes), discomforts and risks that may arise as well as possible alternatives to the proposed treatment including no treatment. The risks of no treatment have also been discussed. I have been given an opportunity to ask questions and all my questions have been answered fully and satisfactorily.

3. I understand that during the course of the procedure(s), unforeseen conditions may arise which necessitates procedures different from those contemplated. I, therefore, consent to the performance of additional procedures that the above-named dentist or his/her associate may considernecessary.

4. I acknowledge that no guarantee or assurances have been made to me concerning the results intended from the procedure(s).

5. No identity lies with the doctor in case the treatment is discontinued or the outcome is not aspredicted.

6. I confirm that I have read and fully understand the above and that all blank spaces have been completed prior to my signing. I give my consent that this record can be used for research, education, training & upliftment of the dentalprofession.

Signature of patient

Date:

HEAD OF DEPARTMENT

ANNEXURE III
CONSENT FORM (HINDI)

सन्तोष डेन्टल कॉलेज

प्रताप विहार, गाजियाबाद

सहमति पत्र

1. मैं .....पुत्र/पत्नी.....निवासी.....

..... अपने ऊपर किये जाने वाले निरीक्षण/ परीक्षण, चिकित्सीय सलाह/पद्धति की पूर्ण सहमति देता/देती हुं।

2. मुझे चिकित्सा प्रणाली और चिकित्सा के उपरान्त होने वाले संभावित नतीजों के बारे में दंत चिकित्सक के द्वारा अच्छी तरह समझाया गया है।

3. मुझे चिकित्सा के दौरान अथवा उसके उपरांत होने वाली संभावित/ अप्रत्याशित हानियों, इलाज के समय उपयुक्त होने वाली दवा व उसके प्रयोग के बारे में भी अच्छी तरह बताया गया है।

4. रोगी के द्वारा चिकित्सा के बीच में ही छोड़कर जाने अथवा चिकित्सा के उपरान्त परिणाम के उम्मीद के अनुसार न आने पर, चिकित्सक की जिम्मेदारी नहीं होगी।

5. मैं पुनः अपनी सहमति देता हूँ कि इस रिकार्ड को भविष्य में चिकित्सीय शोध, प्रशिक्षण एवं चिकित्सा प्रणाली के उत्थान के लिये प्रयोग किया जा सकता है।

रोगी के हस्ताक्षर

तारीख:

ANNEXURE IV
CASE HISTORY SHEET

SANTOSH
Deemed to be University
(Established u/s 3 of the UGC Act, 1956)

Department of Conservative Dentistry and Endodontics

CASE HISTORY RECORD

OPD No :-	Date:-
Patient Name	Gender/Age
Address	Phone No:-
Chief Complaint	
History of Presenting Illness	
Past Dental History	
Past Medical History	
Oral Examination	
Diagnosis	
Treatment Advised	

ANNEXURE V
MEMORANDUM OF UNDERSTANDING (MOU)

Date: 06/09/2024

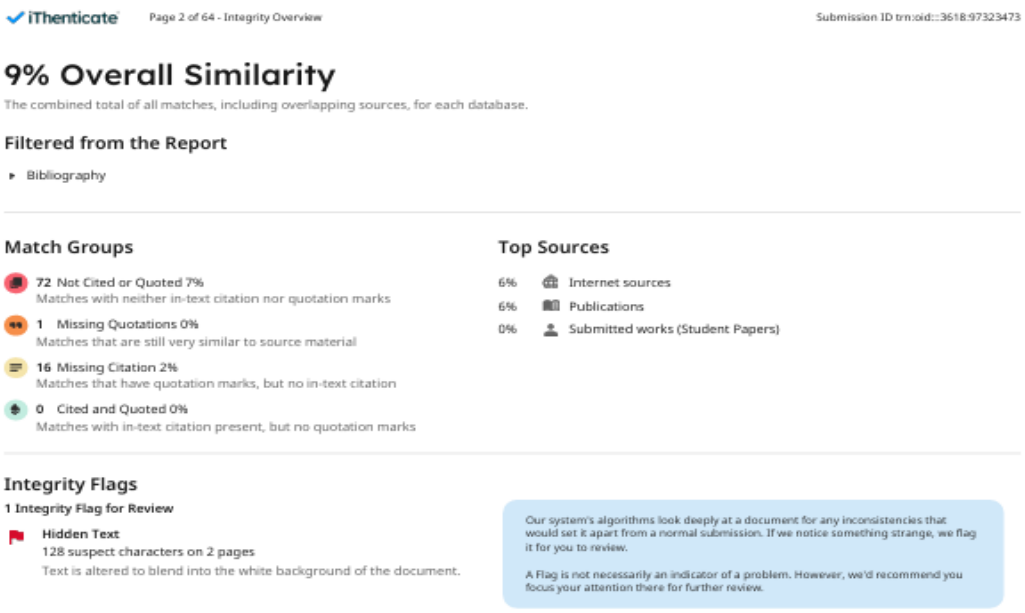
TO WHOMSOEVER IT MAY CONCERN
(MEMORANDUM OF UNDERSTANDING)

This is to certify that **Dr. Ashmita Bhardwaj**, M.D.S (Department of Conservative Dentistry and Endodontics) student from Santosh Dental College & Hospital, No. 1 Santosh Nagar, Ghaziabad, - 201009, NCR, Delhi has performed the study named **"Comparative Evaluation of Antimicrobial Efficacy of Fosfomycin and Calcium Hydroxide with Chlorhexidine Gel (2%) against E.Faecalis in Retreatment Cases using Colony Forming Units – A Randomized Control Trial"** in collaboration with us for her thesis work. The streaking and culture of bacteria on media plates, along with their incubation and CFU study were done at our centre at Pioneer Centre of Biosciences for Advanced Training and Research, Mohan Nagar, Ghaziabad, Uttar Pradesh

Dr Manish Kumar Sharma
Director
Pioneer Centre of Biosciences
Ghaziabad

Dr. Sumita Giri
Professor and Head
Department of Conservative Dentistry and Endodontics
Santosh Dental College and Hospital
Ghaziabad

ANNEXURE VI
PLAIGARISM REPORT



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